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Published Version:
Cesare Accinelli, H.K.A. (2021). Minimizing abrasion losses from film-coated corn seeds. JOURNAL OF CROP IMPROVEMENT, 35(5), 666-678 [10.1080/15427528.2020.1861156].

Published:
DOI: http://doi.org/10.1080/15427528.2020.1861156

*This is an original manuscript of an article published online by Taylor & Francis in **Journal of Crop Improvement** on on 21 Dec 2020 and available at http://www.tandfonline.com/, DOI https://doi.org/10.1080/15427528.2020.1861156*

Minimizing abrasion losses from film-coated corn seeds

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ABSTRACT

Seed film-coating is an emerging technology for precision applications of plant protection agents, beneficial microorganisms, and other substances, to seeds of important agronomic species. During seed handling and planting operations, a variable number of coat fragments are detached by mechanical abrasion and thus released in the environment. Until now, efforts for reducing abrasion losses by selecting and/or developing polymers and formulations to improve adherence on seed surfaces have been achieved limited success.

This study showed that the risk of abrasion losses from film-coated corn seeds was minimized by removing the outer wax layer of the seed pericarp, before applying coat formulations. This additional step improved adherence strength of the coat to the seed surface and abrasion losses were thus effectively reduced. Using three different coat slurries, including a commercial polymer formulation, a starch-based bioplastic, and soy protein isolate-based formulation, fragment release was reduced up to 97.6, 94.8, and 98.9%, respectively. Removal of the outer wax layer did not affect seed germination and seedling growth. However, in addition to stimulating seedling growth, the latter formulation rapidly deteriorated in soil. More precisely after 6 days of incubation in soil, coats from the commercial and the bioplastic formulations showed a deterioration of 2.5, and 72.1%, respectively, while film-coat of the soy protein isolate-based formulation deteriorated almost completely within 6 days.

Keywords

Seed treatment, seed film-coating; biodegradable plastic; microplastic; seed coat abrasion; dust-off; maize; biochar

1. Introduction

Seed treatment with pesticides is a well-established technique that is routinely applied to a large variety of crop and vegetable species to protect germinating seeds and seedlings from seed- and soil-borne pathogens and insects. (Pedrini et al., 2017; Hitaj et al., 2020). In the last decades, with the availability of systemic and high effective pesticides, seed pesticide application has become a predominant practice for assuring high percentages of healthy and uniform seedlings, using less amount of active ingredients than with in-furrow and especially with broadcast applications (Hairston, 2013; Halecky et al., 2016). In corn, and other agronomic species, pesticides are applied by covering the seeds with a thin adherent plastic-like film-coat, consisting of pesticides, polymers, binder and plasticizer agents for also improving seed flowability. Pesticide-treated seeds are then typically colored with pigments for avoiding unintended use as food and feed (McGee, 1981; Aveling et al., 2013; Accinelli et al., 2018a). Recent evidences have shown that even with advancements in seed treatment technologies and polymer science, release of coat fragments by abrasion of film-coated seeds during handling and planting operations remains a matter of concern (Halecky et al., 2016; Accinelli et al., 2018a, 2019). Although this aspect has gained more relevance with the recent increase use of neonicotinoid insecticides in film-coating of agronomic seeds, including corn, and the potential association with bee colony mortality and other environmental issues, only a limited number of studies have focused on estimating the amount of coat fragments that are detached from film-coated seeds (van der Sluijs et al., 2013; Rhodes, 2018; Foqué et al., 2014, 2017; Devarrewaere et al., 2015; Hoffmann et al., 2015). These studies have indicated that during corn planting operations the release of coat fragments can varying from 0.88 to 1.11 g per hectare. Data were generated using the Heubach dustmeter approach, which is widely adopted by pesticide manufacturers, and seed and pesticide industry associations in many countries. This laboratory and gravimetric-based approach is specifically designed for measuring dust-off from pharmaceuticals and food tablets, and other similar items, and more recently, as stated above, it has also been adopted for estimating abrasion losses from coated seeds. When applied for this purpose, a mass of 500 g of seeds is tumbled at a constant speed into a metal rotating drum and purged with air for 30 minutes. Detached coat fragments are thus carried away by the pressurized air stream and captured on paper filter, weighted, and expressed as grams per 100 kg of seeds or per 10,000 seeds (Zwertvaegher et al., 2016; Accinelli et al., 2018b). However, considering that application of film-coat do not significantly alter the weight of seeds, with differences that do not exceeded 1-5 %, most of the frequent discrepancies among predicted dustmeter values and observed effects (i.e., mortality of bee colonies in surrounding cropped areas), likely suggest that more specific approaches and studies are needed to better understand and to estimate the extent of seed abrasion (SA) processes (Hairston 2013; Accinelli et al., 2018a).

Based on their small dimensions, and physico-chemical properties, these detached plastic-like film fragments are conveniently categorized as microplastics (MPs), and more precisely as microplastic coating films (MPCFs) (Accinelli et al., 2019). The term MP refers to plastic particles passing a 5-mm sieve, that are either generated by fragmentation of larger pieces of plastic or those that are directly produced as plastic-based beads or granules (e.g. cosmetics and detergents as abrasives, etc.) (Thompson et al, 2004).

Most of the commercial film-coating formulations that are currently available in the market are produced with proprietary polymers and other unspecified chemicals and mixtures, and very little information on their resistance to abrasion and fate in the environment (i.e., soil) is available in the literature (Pedrini et al, 2017). Recent studies have shown that air dispersion and persistence in soil of detached MPCFs can be reduced by using bio-based and biodegradable formulations with improved adherence to seed surface (Accinelli et al., 2018a, 2019). Film-coating of corn seed is typically achieved using aqueous slurries which are applied to seeds using rotary drum machines. Although, irregularities in the shape of corn kernels are expected to negatively impact the uniformity of film-coat, the external wax layer and its slipperiness is likely to the seed surface.

The main objective of the present study was to investigate a novel approach for reducing the risk of abrasion of film-coated corn seeds. The study also evaluated the feasibility of using an improved image-based protocol for a rapid and effective evaluating of SA.

2. Materials and methods

2.1. Film-coating of seeds

Experiments were carried out using seeds of two corn hybrid varieties, the hybrid PR31D24 (PR) and the hybrid Kenobis (KE), which were provided by Pioneer Hi-bred Italia s.r.l. (Cremona, Italy) and Società Italiana Sementi s.p.a. (Bologna, Italy), respectively. Seeds were treated with three different aqueous coating formulations, a commercial coating polymer formulation (CPF) marketed with the brand name Sepiret (Basf SE, Ludwigshafen Germany), a starch-based liquid bioplastic (BPF), as described in Accinelli et al. (2018a), and a novel formulation containing soy protein isolate and other bio-based ingredients (SBF). More specifically, the latter formulation was prepared by dissolving soy protein isolate (5.3%; w/v) in milli-Q water with pH adjusted to 9.0. After mixing for 30 min. at room temperature, glycerol (10% w/v; reagent grade, ≥ 99.0%) was added and the dispersion was mixed at 85 °C for additional 30 min. Extruded starch (5.5%; w/v), Arabic gum from acacia tree (2.7% w/v), soybean lecithin (0.3% w/v;), soy wax (0.05% w/v) were then added to the dispersion, which was mixed at 40 °C for 20 min. All chemicals were provided by Sigma-Aldrich KgaA (Darmstadt, Germany). As for the BPF, slurry of the SBF was colored with the dye Color Coat Red (Becker Underwood, Ames, Iowa) and heated in a microwave at 450 W for 2 min. An additional soy protein isolate-based formulation was included in this study, which differed by the addition of 2.5% (w/w) of a biochar powder (size < 0.2 mm) (SBF-BC). Harwood biochar was provided by Rockwood Recycling LLC (Lebanon, TN, USA). The different formulations were applied at the rate of 15 mL kg^{-1} of seeds, using a laboratory bench-top rotating drum machine equipped with three 10-cm-wide plastic deflectors (Accinelli et al., 2018a).

The same film-coat treatments were repeated with seeds that were surface grinded for removing the outer wax layer of seed pericarp. Briefly, a mass of 25 g of seeds were transferred in 50-mL tubes centrifuge tube containing 5 g of angular silica bead (average

diameter of 3 mm). Tubes were secured in horizontal position in a modified Vortex Genie 2 (Fisher Scientific, Waltham, MA) equipped with a flat-bed adapter holding 6 tubes and vortexed at maximum speed for 5 min. Surface dewaxed (SD) seeds were then separated from silica beads and pericarp wax residues
using a 4-mm sieve and cleaned with compressed air.

2.2. Measurements of seed film-coat abrasion

The potential of treated seeds to release MPCFs when being subjected to mechanical abrasion forces was measurement following a modification of the procedure described in Accinelli et al. (2018a). Briefly, 25 g of corn seeds were transferred into a 3.0-cm wide and 10-cm height metal cylinder equipped with six 0.5-mm height conical studs (Figure 1). The cylinder was secured in vertical position into an oscillating housing provided with an air flow generator (upper base of the cylinder) and a 2-mm size copper nets was fixed to the lower base of the cylinder. The cylinder was shacked at high speed by an electric engine mounted in the housing. After 10 min., shacking velocity was reduced, air flow was activated, and detached MPCFs were collected in a same dimension plastic centrifuge tubes mounted into a metal base, which was equipped with to an electrostatic charging generator operating for 5 min. The two cylindrical tubes were separated and 25 mL of 4% polyvinyl acetate solution were transferred into the plastic tube containing MPCFs and then triplicate 5-mL aliquots of the dispersion were poured onto 9.0-cm diameter and 2-mm height plastic plates and incubated at 40 °C for 1 h. Three randomly selected 1-cm diameter areas were defined on each polyvinyl acetate film and the MPCFs in them counted. MPCFs were enumerated and sizes calculated by taking detailed images of the three selected areas with a magnification of 10-30× using a smartphone (iPhone 11, Apple Co., Cupertino, CA) mounted on a dissecting microscope. Images were analyzed using the software imageJ version 1.5Oi (National Institutes of Health, Bethesda, MD). Seed abrasion (SA) was expressed as the percentage of the total area of visualized MPCFs per seed.

2.3. Dissipation of seed film-coat samples in soil

The four seed film-coat formulations were also tested for their dissipation in soil. Films of rectangular shape (2,8 cm x 6,0 cm and 15-µm thin) were obtained by casting the three formulations in Teflon molds. After drying at 40 °C for 30 min., the rectangular films were placed between two polyethylene nets with mesh opening of either 5-mm square, secured by hot-melt glue. Assembled rectangular films were then secured in centrifuge tubes (3.1 cm diameter x 11.5 cm long) containing 50 g of 4-mm sieved topsoil (0-10 cm depth) that was collected from an uncropped area at the experimental farm of the University of Bologna, Italy. Soil was classified as a silty loam (Udic Ustochrepts, fine silty, mixed, mesic) with 380 g kg-1 sand, 245 g kg-1 clay, 375 g kg-1 silt, 8.5 g kg-1 organic C, and pH (1:2.5 soil/water mixture) of 8.0. Soil moisture of was adjusted to the field capacity and samples were incubated at 25 °C in the dark in a ventilated incubator for 6 days. Soil moisture was monitored daily and water added as needed. At selected intervals, samples were removed and processed for film degradation evaluation under a dissecting microscope equipped with a Nightsea Fluorescence Adapter (Electron Microscopy Sciences, Hatfield, PA). Images were analyzed using the software imageJ version 1.5oi and film degradation was calculated considering the total area of visible lacerations and holes, with respect to the exposed surface area of single squares. Measurements were done considering the single 6 central 5-mm^2 grid areas.

2.4. Seed germination and seedling growth

Coated seeds were evaluated for their germination and seedling growth. For each treatment, 100 seeds of each hybrid were randomly selected and placed between rolled, moist paper towels (10 seeds per roll) and incubated in a germination chamber at 20 °C (80% relative humidity) with 12 h of light per day. Germination percentage was recorded daily and mean germination time (MGT) calculated as following: MGT = $\Sigma(n_i\, d_i)/\Sigma n_i$, where n_i is the number of seedlings present on day i, and d_i is the number of days since the beginning of the test (Ellis and Roberts, 1980).

Seeds from the same batches were then planted in soil pots and placed in a growth chamber for evaluating seedling growth. Plastic pots (18-cm diameter; 25-cm high) were filled with 350 g of 4-mm sieved topsoil (0-10 cm depth) that was collected from an uncropped area at the experimental farm of the University of Bologna, Italy. Soil was classified as a silty loam (Udic Ustochrepts, fine silty, mixed, mesic) with 380 g kg^{-1} sand, 245 g kg^{-1} clay, 375 g kg^{-1} silt, 8.5 g kg^{-1} organic C, and pH (1:2.5 soil/water mixture) of 8.0. Soil was homogenized by passing through a 4-mm sieve, and the moisture was adjusted to the field capacity. Pots were planted with 2 seeds each at a depth of 3 cm and maintained in a growth chamber for 21 days under supplemental light for a 12-h period with day and night temperatures of 25 °C and 15°C (80% relative humidity), respectively. Soil was watered daily, and after a 10-day period, seedlings were carefully removed, washed under running tap water to remove adhering soil, dried in a ventilated oven for 48 h, and dried weight recorded. For each treatment, 100 seeds were plated in pot soil. Germination percentage and MGT were determined as above.

2.5. Statistical analysis

Seed abrasion tests were conducted in triplicate. For each experimental treatment, seed germination and seedling growth were evaluated on a total of 100 randomly selected seeds.

Means were separated by Fisher's least significant difference (LSD), and P values <0.05 were considered statistically significant.

3. Results and discussion

3.1. Seed film-coat abrasion

In the present study, surface abrasion (SA) of film-coated seeds was evaluated using a visual-based approach. Considering that film-coated seeds are artificially colored and that detached fragments are more likely to resemble microplastic films (MPFs) than dust particles, previous studies have thus shown that SA is conveniently evaluated following this visual approach, instead of using the Heubach dustmeter, which is based on measuring their mass (Accinelli et al., 2018a, 2019). With this method, abrasion is generated by vigorously shaking seeds into tubes provided with abrading studs. Detached MPFs are electrostatically charged and thus easily separated from seeds. MPFs are then visualized and total area automatically determined (Accinelli et al., 2008a). As shown in Figure 1, film-coated seeds showed a variable resistance to mechanical abrasion. Seeds that were coated with the CPF exhibited a significantly lower ($p < 0.05$) resistance to abrasion than those with the starch-bioplastic formulation (BL). More specifically, when CPF and BPF were used for film-coating of seeds of the PR hybrid, SA values were of 119.2 and 24.4 mm^2 per seed, respectively. These differences were consistent with those observed with the KE hybrid. In this case, SA was of 124.4 and 20.9 mm^2 per seed, with the CPF and BPF, respectively.

Working with lots of commercial corn seeds and seeds of other crop species from different European countries, authors have reported a large variability of their resistance to abrasion (Foquè et al., 2014). Results were explained considering differences in the quality of film-coat treatments, which includes accuracy of seed treatment equipment and feasibility of coat formulations, with no specific information concerning shape and size of seed species and varieties. However, these aspects, including physico-chemical properties of external seed layers, are likely to affect adhesion strength of film coats on seed surfaces. For instance, regardless of type of film-coat formulation, authors have reported higher SA values with corn than with regular spherical-shaped seeds (e.g. canola and soybean seeds) (Accinelli et al., 2018a). In addition, corn seeds are typically covered by a waxy layer, whit differences among hybrids (e.g., amount, uniformity of coverage) (Mellon and Moreau, 2004). The amount of recovered wax from the surface of PR and KE hybrids was of 0.81 and 0.79 mg per seed, respectively. Since the outer wax layer is expected to reduce adhesion against aqueous and hydrophilic film-coating formulations, such as those used in this study, the observed reduced differences in terms of recovered wax were consistent with SA values of the two hybrid seeds.

As in other film-coating industrial applications, in seed film-coating, seeds are coated by covering their entire surface by a thin plastic-like thin film. Findings reported here further supported that the starch-based BPF was effective in reducing SA (Accinelli et al., 2018a). In the present study, chitin was excluded from the original formulation for reducing stiffness of the final plastic-like coat, and, as expected, this resulted in higher adhesion to seed surfaces. Other modifications were also explored. Although some combinations (i.e., addition of rosin and/or methylcellulose) further improved resistance to coat abrasion, these solutions negatively impacted seed flowability and/or germinability (data not shown). These preliminary experiments suggested that the search for other polymers or resins with improved adherence to the smooth and wax-covered surface of corn seeds would not necessarily lead to effective practical solutions. Investigations were consequently directed to a different approach, which consisted in removing the thin external waxy layer of seed pericarp. Surface dewaxed (SD) seeds were thus film-coated using different formulations and SA measured. As shown in Table 1, removal of the external waxy layer lead to a significant reduction (p <

0.05) of SA, with reductions up to 94.8%. With the CPF, average SA value was of 2.9 mm^2 per seed, with no significant differences ($p > 0.05$) among the two hybrid seeds. This was also observed with BPF, which showed SA that did not exceeded 1.3 mm^2 per seed. Given these positive results, SD seeds were also coated with a formulation prepared with accessible and readily water-soluble ingredients. Although this formulation, that was prepared with dissolve soy protein isolate and other natural ingredients (SBF), was not effective in adhering to the surface of non-SD seeds, when the waxy layer was removed, it showed high resistance to abrasion, with SA values comparable to those observed with the BPF. Addition of biochar powder lead to a slight decrease ($P > 0.05$) of SA, which was likely due to the reduction of elongation properties of the film coating.

3.2. Seed germination, seedling growth and MPF degradation in soil

A major aspect to take into accounts in developing novel seed coating solutions consists in evaluating their potential effects on seed germination and seedling growth. Results of the paper towel germination assay are shown in Table 1 and 2. Coating the seeds of the two corn hybrids with either CPF, BP, and the SBF, had no effect ($p > 0.05$) on germination percentage. Germination remained also unaffected ($p > 0.05$) when the thin external waxy layer was removed. However, these surface dewaxed (SD) seeds showed a slightly lower mean germination time (MGT) ($p > 0.05$) than the control. This shortening of germination time was not observed when SD seeds were then coated with either the different tested formulations. Removal of the hydrophobic waxy layer was likely to results in a slight reduction of the time required for seed imbibition and thus accelerating seed germination. Intense scarification of seed integuments is commonly adopted with several wild species (i.e., weeds), horticultural species and other dormant seeds for interrupting dormancy and inducing germination (Elias et al, 2012). Seed scarification is either achieved by mechanical (i.e., clipping, piercing, etc.) or chemical (i.e., use of concentrated sulfuric acid) procedures. No information on effects of the moderately invasive removal of the external waxy layer on germination of corn seeds and other species is reported in the literature. Studies have shown that film-coating has a little, or no effects on seeds germination. When negative effects are observed, these are mainly due to slight residual phytotoxic effects of active ingredients, which, in most cases, only occur when applied doses are above those indicated by pesticides manufactures (Taylor and Selanenka, 2012; Keawkham et al., 2014).

Seed germination tests were repeated in soil pots. As shown in Table 1, results were comparable to those that were observed in the paper towel test. However, SD seeds that were coated with the SBF resulted in significantly greater ($p < 0.05$) seedling dry biomass. The stimulatory effect of soy proteins, and more in general of plant proteins, on seedling growth has been recently reported by other authors (Amirkhani et al., 2016, Colla et al., 2014). In these studies, when seeds of corn, tomato, and broccoli, were coated with aqueous slurries containing protein isolates, N uptake, enzyme activity, and other metabolic processes were improved. The stimulatory effect on seeding growth was not affected ($p > 0.05$) when biochar powder was incorporated into the SBF. In this study, the addition of this renewable material was included for evaluating the feasibility to enrich this bio-based formulation with nutrients and/or biochar-sorbed plant protection active ingredients. In addition, it was observed that addition of biochar powder to the film coat slurry discouraging wild birds from eating these seeds (data not shown).

In the present experiment, the potential of the three film-coating formulations to deteriorate in the soil was also investigated. As shown in Figure 2, MPFs obtained from the CPF showed very little deterioration with respect to those from the other two formulations. More specifically, at the end of the 6-day incubation period, deterioration of MPFs from CPF, BPF, SBF, and SBF-BC accounted for 2.5, 72.1, 98.0% and 97.2%, respectively. Either the BPF and the SBF formulations was obtained from bio-based compounds (e.g., destructured starch, soy protein isolates). In addition, SBF was specifically designed to include ingredients with higher water solubility for promoting a rapid microbial degradation of MPFs in soil.

4. Conclusions

Despite of recent improvements in seed film-coating technologies and polymer science, detachment of coat fragments by mechanical abrasion occurring during seed handling and planting operations is still a matter of concern, especially for irregularly shaped seed species. This series of study demonstrated the feasibility of minimizing abrasion losses by including an additional step, consisting in the removal of the outer wax layer of seed pericarp, before proceeding with conventional seed film-coating processes. Application of this additional step had no effects on seed germination and seedling growth. In contrast, using different coating formulations, including a commercial polymer formulation and two bio-based formulations, abrasion losses were reduced up to 94.8%, with respect to non-surface dewaxed seeds. This approach opened the possibility of using novel coating solutions, especially bio-based and rapidly biodegradable in soil.

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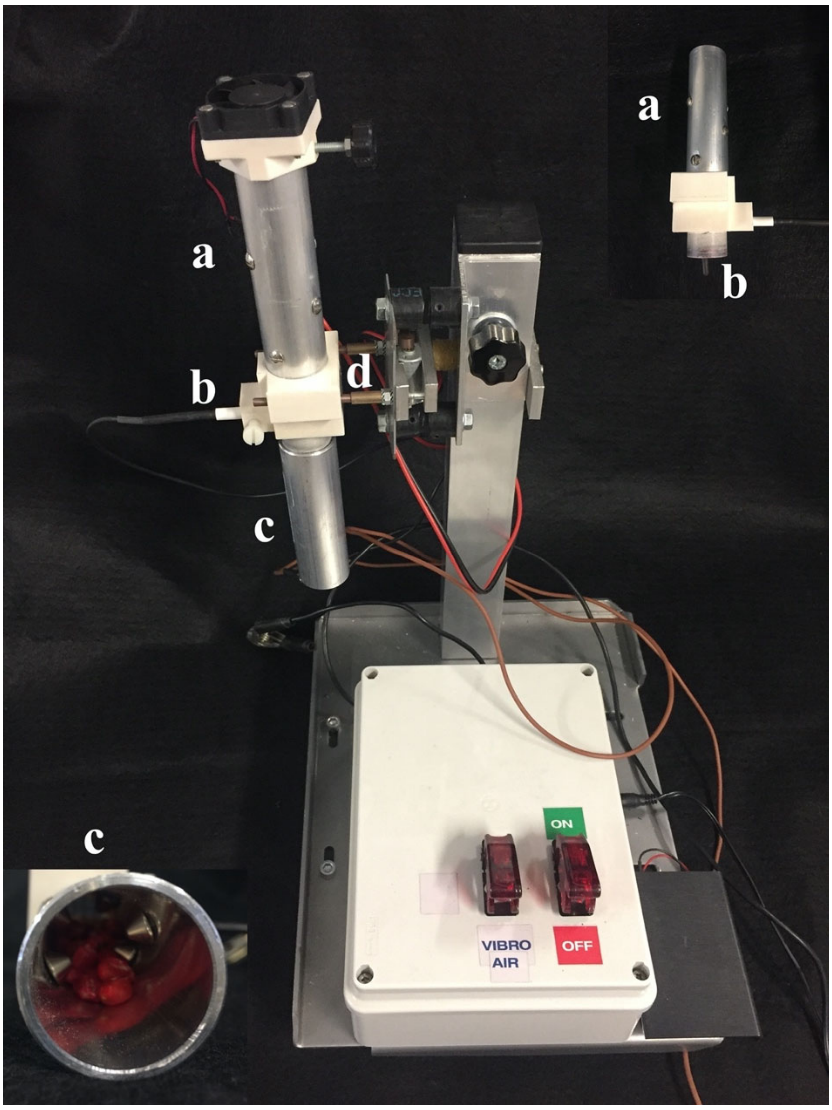

Figure 1. Apparatus for the measurement of seed abrasion; a) abrasion cylinder with conical studs; b) electrostatic probe; c) fragment collecting cylinder; d) agitation mechanisms.

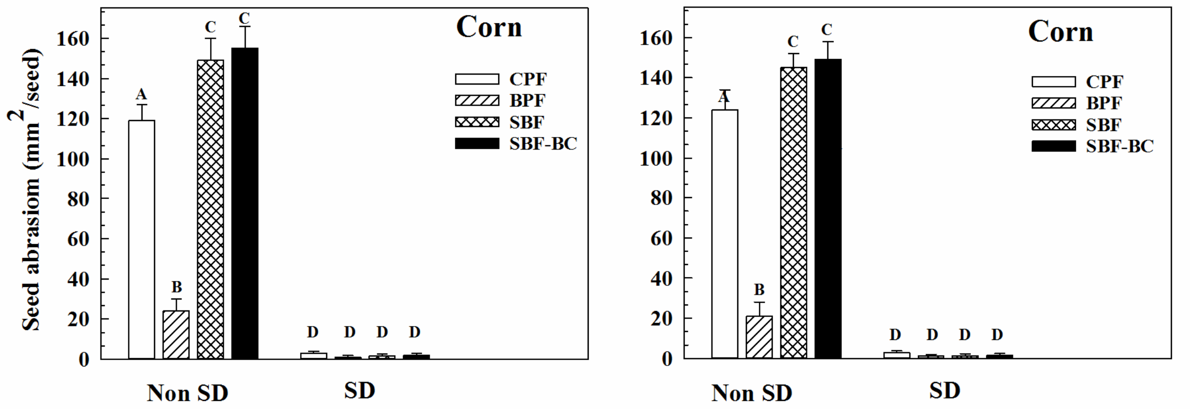

Figure 2. Effect of the removal of the outer waxy layer of seed pericarp on seed abrasion (SA). Seeds were surface dewaxed (SD) and then coated with a commercial polymer (CPF), a corn starch-based bioplastic (BPF), a bio-based formulation containing soy protein isolate and other ingredients (SBF) or a SBF with the addition of biochar (SBF-BC). SA is expressed as percent of total area of detached film coat fragments per seed. Measurements were carried-out with seeds of two hybrids (PR: PR31D24, KE: Kenobis). Bars with same letter are not significantly different ($P > 0.05$).

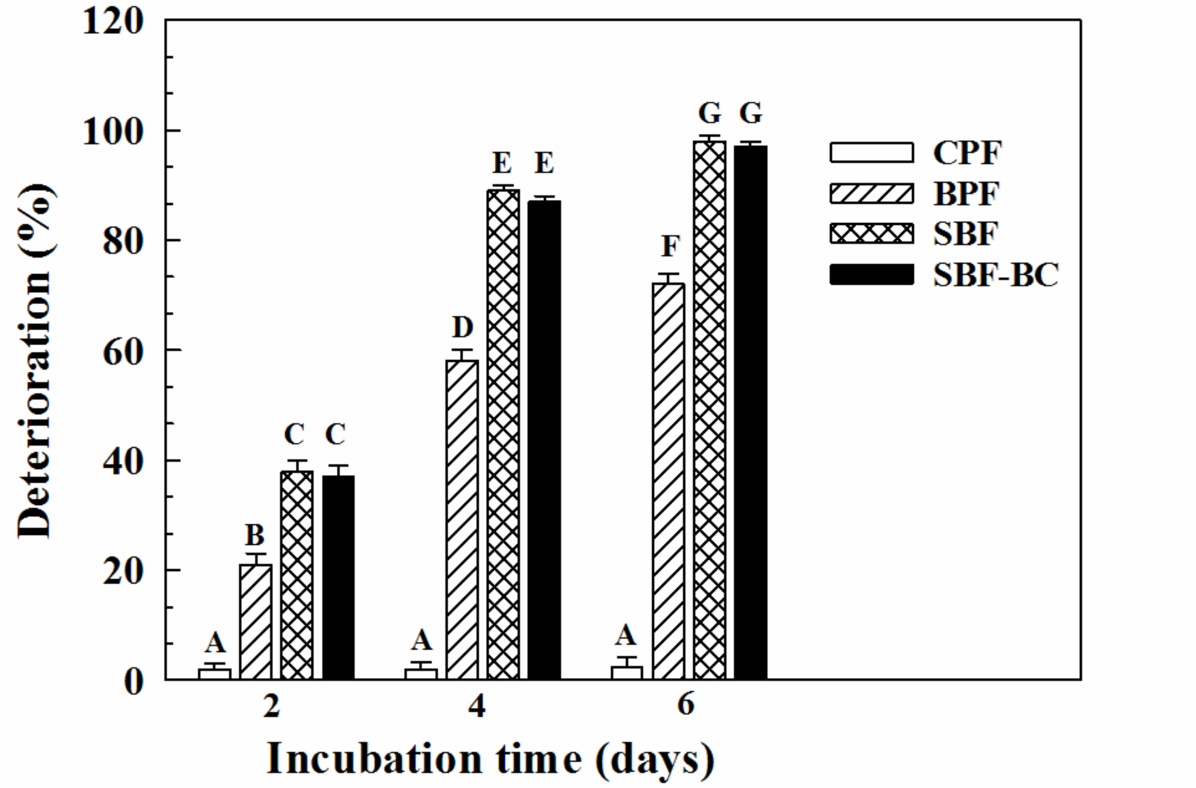

Figure 3. Deterioration of micro-sized (5 mm) film-coat in soil. Film-coat samples were obtained by film casting the four different formulations that were used for coating corn seeds, namely, a commercial polymer (CPF), a corn starch-based bioplastic (BPF), a bio-based formulation containing soy protein isolate and other ingredients (SBF) or a SBF with the addition of biochar (SBF-BC). Bars with same letter are not significantly different ($P > 0.05$).

Table 1. Effect of the removal of the outer waxy layer of seed pericarp on percent germination, mean germination time and seedling dry mass. Surface dewaxed (SD) and non SD seeds were either uncoated or were coated with commercial polymer (CPF), a corn starch-based bioplastic (BPF), a bio-based formulation containing soy protein isolate and other ingredients (SBF) or a SPF with the addition of biochar (SBF-BC). SA was expressed as a percent of total area of detached film coat fragments per seed. Measurements were carried-out with seeds of the corn hybrid PR31D24. Bars with same letter are not significantly different (P > 0.05).

	Germination (%)		Mean germination time (%)		Seedling dry weight (g)
Non SD - Uncoated	99.02 a	98.06 a	3.61 a	3.70 a	0.35 a
SD - Uncoated	98.09 a	97.98 a	3.12 a	3.08 a	0.32 a
Non SD - CPF	97.99 a	99.01 a	3.75 a	3.61 a	0.30 a
SD - CPF	98.12 a	98.07 a	3.80 a	3.76 a	0.32 a
Non SD - BPF	98.83 a	98.97 a	3.68 a	3.59 a	0.31 a
SD - BPF	99.02 a	99.00 a	3.73 a	3.61 a	0.33 a
Non SD - SBF	97.97 a	98.91 a	3.66 a	3.71 a	0.41 b
SD - SBF-BC	98.03 a	98.07 a	3.70 a	3.66 a	0.43 b

*Values within a column followed by the same letter are not significantly different (P > 0.05) (LSD).

Table 2. Effect of the removal of the outer waxy layer of seed pericarp on percent germination, mean germination time and seedling dry mass. Surface dewaxed (SD) and non SD seeds were either uncoated or were coated with commercial polymer (CPF), a corn starch-based bioplastic (BPF), a bio-based formulation containing soy protein isolate and other ingredients (SBF) or a SPF with the addition of biochar (SBF-BC). SA was expressed as a percent of total area of detached film coat fragments per seed. Measurements were carried-out with seeds of the corn hybrid Kenobis. Bars with same letter are not significantly different (P > 0.05).

	Germination (%)		Mean germination time (%)		Seedling dry weight (g)
Non SD - Uncoated	98.08 a	98.24 a	3.70 a	3.66 a	0.30 a
SD - Uncoated	98.33 a	99.01 a	3.09 a	3.11 a	0.35 a
Non SD - CPF	98.51 a	98.17 a	3.81 a	3.73 a	0.33 a
SD - CPF	97.32 a	98.20 a	3.79 a	3.69 a	0.35 a
Non SD - BPF	98.21 a	97.99 a	3.71 a	3.72 a	0.34 a
SD - BPF	98.35 a	98.11 a	3.68 a	3.76 a	0.03 a
Non SD - SBF	98.45 a	98.70 a	3.74 a	3.74 a	0.46 b
SD - SBF-BC	98.10 a	97.90 a	3.67 a	3.70 a	0.48 b

*Values within a column followed by the same letter are not significantly different (P > 0.05) (LSD)