THE INFLUENCE OF PDA, CMA, TA AND V8 CULTURE MEDIA ON THE DEVELOPMENT OF *PHYTOPHTHORA INFESTANS* DE BARY. AND *PHYTOPHTHORA PARASITICA* DAST.

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Abstract

The aim of the study was to learn the influence of culture media on two species of *Phytophthora*. The development and colony appearance was observed for ten days and the number of spores for ten weeks. In order to achieve this experiment two species of the genus *Phytophthora* were used *Phytophthora infestans* de Bary and *Phytophthora parasitica* Dast. and four culture media PDA, CMA, V8 and TA. In determining the number of spores, 63.63 cm^2 of mycelium were collected from the surface of the culture medium and mixed with 250 ml of sterile water. The containers with sterile water and *Phytophthora parasitica* Dast. and *Phytophthora infestans* de Bary., were placed in a refrigerator at a temperature of 7° C, to make sure that zoospores are released. The number of spores per milliliter was determined for each of the fungi using the hemocytometer. The highest values were recorded on the dishes with PDA medium, where after calculating the average of nine dishes the colony registered a rate of growth of approximately 1.47 cm. According to these observations we can conclude that the PDA is the most favorable for the development of the *Phytophthora infestans* de Bary colony. The highest values of the *Phytophthora parasitica* Dast colony diameter were observed in the plates with V8, where more than half of the plates have reached the edge of the analyzed Petri plates, thus an average diameter of 8.4 cm was registered in the last day of observation. On the TA plates there was also a high growth rate, the diameter of the colony reaching an average value of 5.5 cm at the end of the 10^{th} day of observation. The PDA provided a slower growth rate of the colony diameter, at the end of the observations the colony only measured 3.94 cm, while CMA media composition has provided conditions for development of the average diameter of only 1.67 cm.

Key words: *Phytophthora parasitica*, *Phytophthora infestans*, culture media, number of spores

The origin of the *Phytophthora* genus is closely associated with what is now known as the Great Irish Famine. Initially, this pathogen was called *Botrytis infestans* (Montagne J.F.C. 1845) and then *Peronospora infestans* (Unger F., 1847). Only two decades later, Anton de Bary (Tucker C.M., 1933), a German mycologist, named the blight producing fungus, *Phytophthora infestans*. The genus name is derived from the Greek φυτόν (Phyton) which means the plant and φθορά (phthorá) which means destruction.

With the establishment of the genus name, scientists have begun to describe other species of this genus, such as *Phytophthora cactorum* in 1870 (Lebert H., Cohn F., 1870), *Phytophthora nicotianae* (Tucker C.M., 1933), *Phytophthora phaseoli* (Thaxter R., 1889) and *Phytophthora colocasiae* (Waterhouse G. M., 1970).

The mechanism of infection of pathogens like *Phytophthora* is the same, usually initiated by mobile zoospore, biflagelated, produced in zoosporangia; they form apical hyphae during the asexual sporulation. These zoospores are attracted to hosts by detecting potential chemical or electrical gradients around the plant (van West P. *et al*., 2002).

On the surface of the attacked plants, the zoospores form small cysts and during this step, they secrete in cortical vesicles an adhesive material that helps form a cellulose wall. Germination of these cysts takes 20-30 minutes from its formation (Robold A. V., Hardham A.R., 2005).

During the penetration of the attacked plant epidermis, the apex of the germination tube transforms itself into an appresorium-like structure, separated from the rest of the tube with a septum (Kebdani N. *et al*., 2010).

Colonization in attacked tissues is facilitated by the enzymes resulting from the degradation of cell wall. In the susceptible plant tissue to the pathogen, the infection occurs rapidly in the young roots 3-5 days after inoculation (Wu C.-H. *et al*., 2008).

Phytophthora parasitica Dast. is a polyphagous species is that occurs especially in the

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Mediterranean climate, with temperatures above 27ºC, where the temperature of the soil and humidity are high (Lamour K., 2013). This micromycetes resists in soil under the form of chlamydospores or in infected plant debris.

Phytophthora infestans de Bary. is a polyphagous species that generally attacks, under natural conditions, species of the Solanaceae family, but has been identified on *Ipomoea purpurea*, *Pharbitis nil* and *Rumex acetosa* var. *hortensis* (Vega-Sanchez M.E. *et al*., 2000).

MATERIAL AND METHOD

The aim of the study was to learn the influence of culture media on two species of *Phytophthora*. The development and colony appearance was observed for ten days and the number of spores for ten weeks starting with the 18th of April 2016. While determining the diameter of the colony observations were made daily. in order to determine the number of spores, observations were made once every two weeks.

In order to achieve this experiment two species of the genus *Phytophthora* were used *Phytophthora infestans* de Bary and *Phytophthora parasitica* Dast. and four culture media PDA, CMA, V8 and TA.

Potato-dextrose-agar (PDA) is a common culture media obtained from potato extract, dextrose and agar. In order to obtain 1l of PDA, in 1l of distilled water 39 grams of PDA from Scharlau were added and after homogenization, it was autoclaved at 121ºC for 15 minutes.

Cornmeal Agar (CMA) is a culture media obtained from cornmeal extract and mixed with agar. In order to obtain 1l of CMA, in 1l of distilled water 17 grams of CMA from Mecconti were added and after homogenization, it was autoclaved at 121ºC for 15 minutes.

V8-juice media (V8) is a culture media realized with V8 juice, calcium carbonate ($CaCO_3$) and agar. In order to achieve the V8 juice the juicer was used and the juice from 400 grams of carrots, 200 grams of celery, 400 grams of tomatoes, 250 grams of beetroot, 200 grams of peppers, 200 grams of spinach, 200 grams of lettuce and 100 grams of parsley was mixed. The juice was passed several times through gauze. To obtaining a liter of media 100 ml V8 juice, 15 grams of agar, 3 g of calcium carbonate ($CaCO_3$) were added and then filled with distilled water up to 1 liter. After homogenization the media was autoclaved at 121ºC for 15 minutes (Jeffers S.N., Martin S.B., 1986).

Tomato agar media (TA) is a culture media obtained from tomato juice, calcium carbonate ($CaCO_3$) and agar. In order to obtain 1l of TA media, 100 ml of tomato juice, 15 grams of agar and 3 g of calcium carbonate ($CaCO_3$) were added and then filled with distilled water up to 1 liter. After homogenization the media was autoclaved at 121ºC for 15 minutes (Jeffers S.N., Martin S.B., 1986).

Culture media was poured into Petri plates (Gosselin 3 vents) with a diameter of 9 cm, and then seeded with both *Phytophthora parasitica* Dast. and *Phytophthora infestans* de Bary. After seeding Petri dishes were kept in an incubator at a temperature of 27ºC.

In determining the number of spores, 63.63 cm^2 of mycelium were collected from the surface of the culture medium and mixed with 250 ml of sterile water. The containers with sterile water and *Phytophthora parasitica* Dast. and *Phytophthora infestans* de Bary., were placed in a refrigerator at a temperature of 7° C, to make sure that zoospores are released. The number of spores per milliliter was determined for each of the fungi using the hemocytometer.

RESULTS AND DISCUSSIONS

As a result of the measurements performed, differences were observed both in the colonies development and in the number of spores in each of the two species of *Phytophthora*.

Phytophthora infestans de Bary. recorded an increase of about 0.3 cm in diameter per day on the PDA plates, in average, plates with CMA, TA and V8 media registred an almost insignificant growth of 0.01 cm in diameter per day (Fig.1).

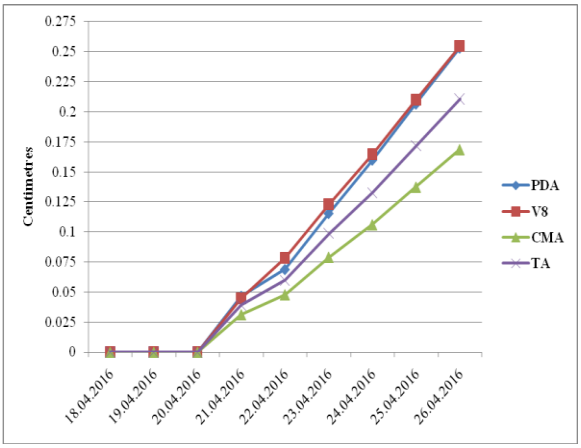

Figure 1 **Development of the *Phytophthora infestans* de Bary. colony diameter (in cm) in the period 18.04.2016-26.04.2016**

Petri plate area is 63.63 cm^2 and at the end of 10 days of observations on the PDA plates, the area occupied by *Phytophthora infestans* de Bary. colony was around 1.7%, on CMA media the values were 0.022% and 0.008% on V8.

Table 1

The growth rate of the colony diameter/day, occupied area (cm^2) and the occupied percentage of plates (%) of the fungus *Phytophthora infestans* de Bary. during 18.04.2016-26.04.2016

Culture media	Growth rate of the colony diameter/day								
	18.04.	19.04.	20.04.	21.04.	22.04.	23.04.	24.04.	25.04.	26.04.
PDA	0	0	0.078	0.222	0.367	0.578	0.811	1.156	1.467
V8	0	0	0	0.067	0.078	0.089	0.089	0.089	0.100
CMA	0	0	0	0.033	0.044	0.078	0.100	0.133	0.167
TA	0	0	0	0.011	0.011	0.011	0.011	0.011	0.011
Culture media	Occupied area (cm^2)								
	18.04.	19.04.	20.04.	21.04.	22.04.	23.04.	24.04.	25.04.	26.04.
PDA	0	0	0.005	0.039	0.106	0.262	0.516	1.048	1.689
V8	0	0	0	0.003	0.005	0.006	0.006	0.006	0.008
CMA	0	0	0	0.001	0.002	0.005	0.008	0.014	0.022
TA	0	0	0	0	0	0	0	0	0
Culture media	Occupied percentage of plates (%)								
	18.04.	19.04.	20.04.	21.04.	22.04.	23.04.	24.04.	25.04.	26.04.
PDA	0	0	0.007	0.061	0.166	0.412	0.812	1.648	2.591
V8	0	0	0	0.005	0.007	0.010	0.010	0.010	0.015
CMA	0	0	0	0.001	0.002	0.007	0.012	0.022	0.034
TA	0	0	0	0	0	0	0	0	0

The highest values were recorded on the dishes with PDA medium, where after calculating the average of nine dishes the colony registered a rate of growth of approximately 1.47 cm. According to these observations we can conclude that the PDA is the most favorable for the development of the *Phytophthora infestans* de Bary colony.

Table 2

The number of spores determined once every two weeks for the fungus *Phytophthora infestans* de Bary.

Culture media	Number of milion spores/week				
	2	4	6	8	10
PDA	1.79	2.33	1.62	1.31	1.05
V8	0.32	0.35	0.28	0.22	0.20
CMA	0	0	0	0	0
TA	0.25	0.28	0.21	0.18	0.16

Phytophthora infestans de Bary. has sporulated on three of the four culture media (PDA, V8 and TA) the lowest values were recorded on the TA culture media with a maximum of 280 000 spores per ml, followed by 356000 spores per ml on the V8 medium and on CMA media the fungus has not sporulated (Fig. 2).

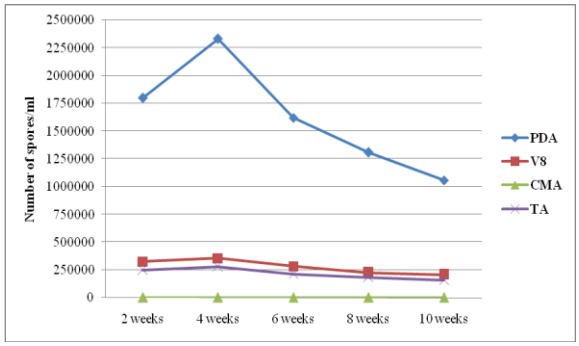

Figure 2 **The chart of spore concetration/ml of *Phytophthora infestans* de Bary.**

As with the colony development, the most favorable media for sporulation was the PDA media. After averaging nine plates, the maximum concentration of spores/ml was observed in four weeks, with a value of 2332000 spores/ml. A significant increase after the first four weeks, followed by decreasing the number of spores was observed on all of the sporulating culture media.

Although there are differences between the concentration of spores drawn from culture media plates with TA, V8 and PDA, according to the literature it requires a concentration of 240 000 spores/ml in order to achieve infection (Fraedrich S. W. et al., 1989). Following these observations it can be concluded that all three culture media are favorable in terms of sporulation.

In terms of appearance the colonies on the four culture media, there were no great differences, the colony was best noted on the PDA medium where they developed the most.

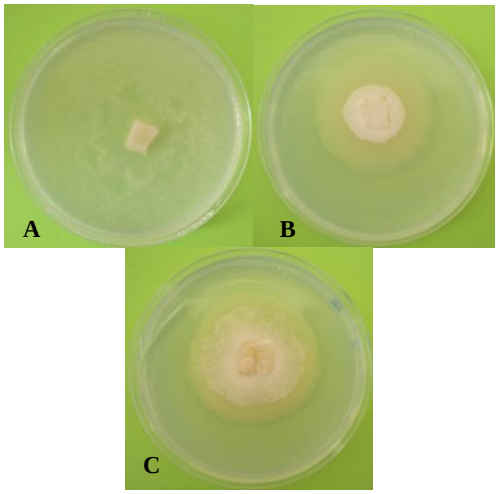

Figure 3 ***Phytophthora infestans* de Bary. colony A- on TA culture media B- on V8 culture media C- on PDA culture media**

In both the case of *Phytophthora infestans* de Bary. colonies developed on PDA and colonies developed on V8, we observed them as having an irregular shape, with a wavy edge and a curved profile. The colonies developed on a culture medium TA, have only very fine hyphae with a filamentous form and edge and a flat profile.

Long simpodial composed sporangiophores with a small thickening in the grip of the sporangia, ranging in size from 100-220 µm were present. Sporangia are lapsed, ovoid, ellipsoidal or lemon shaped, ranging in size from 20-35 X 25-35 µm which provide during germination zoospores or directly mycelium. Oosporii are spherical, colorless, with a size of 30-35 µm. No notice of chlamydospores.

Phytophthora parasitica Dast. recorded an average increase of about 0.6 cm per day on plates with culture media PDA and an increase of 0.9 cm per day on dishes with V8. Plates with CMA in the first three days of observation had an average growth of about 0.5 cm per day, after colony diameter stagnation was noticed followed by a very slow growth up to 0.1 cm per day. Colonies that developed in plates with TA had a steady average growth of 0.7 cm per day.

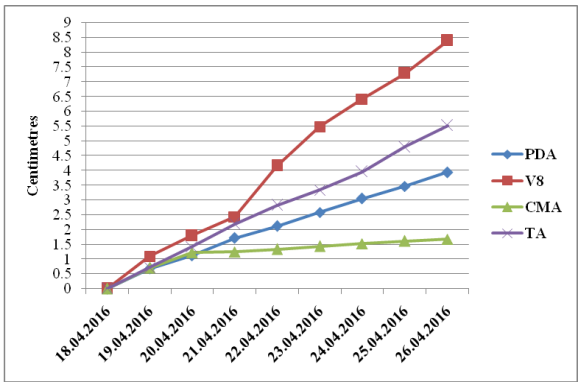

Figure 4 **Development of the *Phytophthora parasitica Dast.* colony diameter (in cm) in the period 18.04.2016-26.04.2016**

Petri dish area is 63.63 cm^2 and at the end of the 10th day of observations on V8 medium, the area occupied by the *Phytophthora parasitica* Dast colony. was around 87%, on TA medium 37.6%, on PDA media the colony occupied 19.2% of the total surface, and on CMA the colony has grown only 3.4% of the Petri plates.

Table 3

The growth rate of the colony diameter/day, occupied area (cm^2) and the occupied percentage of plates (%) of the fungus *Phytophthora parasitica* Dast. during 18.04.2016-26.04.2016

Culture media	Growth rate of the colony diameter/day								
	18.04.	**19.04.**	**20.04.**	**21.04.**	**22.04.**	**23.04.**	**24.04.**	**25.04.**	**26.04.**
PDA	0	0.678	1.122	1.722	2.122	2.589	3.056	3.467	3.944
V8	0	1.100	1.800	2.433	4.167	5.489	6.422	7.289	8.400
CMA	0	0.711	1.222	1.256	1.344	1.444	1.533	1.611	1.678
TA	0	0.722	1.433	2.200	2.822	3.344	3.967	4.800	5.522
Culture media	**Occupied area (cm^2)**								
	18.04.	**19.04.**	**20.04.**	**21.04.**	**22.04.**	**23.04.**	**24.04.**	**25.04.**	**26.04.**
PDA	0	0.361	0.989	2.328	3.536	5.261	7.329	9.434	12.214
V8	0	0.950	2.543	4.648	13.628	23.650	32.377	41.705	55.390
CMA	0	0.397	1.173	1.237	1.419	1.638	1.846	2.038	2.210
TA	0	0.409	1.613	3.799	6.252	8.780	12.352	18.086	23.939
Culture media	**Occupied percentage of plates (%)**								
	18.04.	**19.04.**	**20.04.**	**21.04.**	**22.04.**	**23.04.**	**24.04.**	**25.04.**	**26.04.**
PDA	0	0.567	1.554	3.660	5.558	8.271	11.522	14.831	19.201
V8	0	1.493	3.998	7.307	21.425	37.180	50.900	65.564	87.077
CMA	0	0.624	1.844	1.945	2.231	2.575	2.901	3.203	3.474
TA	0	0.644	2.535	5.973	9.829	13.804	19.418	28.433	37.633

The highest values of the *Phytophthora parasitica* Dast colony diameter were observed in the plates with V8, where more than half of the plates have reached the edge of the analyzed Petri plates, thus an average diameter of 8.4 cm was registered in the last day of observation. On the TA plates there was also a high growth rate, the diameter of the colony reaching an average value of 5.5 cm at the end of the 10th day of observation. The PDA provided a slower growth rate of the colony diameter, at the end of the observations the colony only measured 3.94 cm, while CMA media composition has provided conditions for development of the average diameter of only 1.67 cm.

Table 4

The number of spores determined once every two weeks for the fungus *Phytophthora parasitica* Dast.

Culture media	Number of milion spores/week				
	2	4	6	8	10
PDA	1.686	2.352	2.044	1.68	1.47
V8	1.648	2.554	2.192	2.01	1.886
CMA	1.754	1.898	1.86	1.802	1.578
TA	1.858	2.586	2.464	1.8	1.798

Although the colony of *Phytophthora parasitica* Dast. developed with higher rate on V8 culture media, TA media ensures a more abundant sporulation at the end of the first four-week, the average of the nine culture plates showed a value of 2586000spores/ml. Very close to this value were the plates with V8 2554000 spores/ml, followed by the values recorded on PDA 2352000 spores/ml and CMA with 1898000 spores /ml.

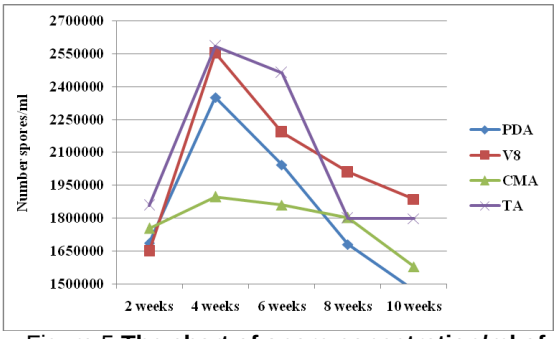

Figure 5 **The chart of spore concetration/ml of *Phytophthora parasitica* Dast.**

According to the literature, a concentration of 240000 spores/ml of *Phytophthora parasitica* Dast. is needed in order to achieve infection (Fraedrich S. W. et al., 1989). Therefore all four culture media are favorable in terms of sporulation, the values obtained are about 10 times higher after four weeks of observation. Just like in the case of the *Phytophthora infestans* de Bary. the number of spores decreases after four weeks of observations.

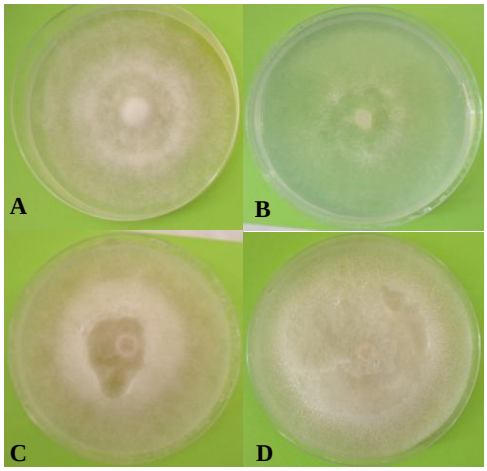

Figure 6 ***Phytophthora parasitica* Dast. colony A- on PDA culture media B- on CMA culture media C- on TA culture media D - on V8 culture media**

PDA developed colonies have a round shape with a whole edge with concentric zonality that is formed during the third week and an umbonate profile. CMA developed colonies had a filamentous form, with a full edge and a flat profile. Colonies developed on TA media had a round shape, filamentous edges and crater form profile. *Phytophthora parasitica* Dast. colony developed on V8 had a round shape with a full edge and a curved profile.

Long sporangiophores with dimensions between 100-300 µm, with spherical or ovoid zoosporangia, papilla, with dimensions between 25-35 µm X 30-45 µm, which during the germination give zoospore or mycelium directly were observed. Zoosporangia are not caduceus. Oospores are spherical, colorless, with dimensions between 25-30 µm. Round chlamydospores with thick brown walls and with dimensions between 20-60 µm.

To investigate the influence of culture medium on the two fungi *Phytophthora parasitica* Dast., and *Phytophthora infestans* de Bary., ANOVA- Single factor test was applied. To confirm the hypothesis that the culture media PDA, CMA, TA and V8 influence both colony growth and the number of spores per ml, P Value must have a value greater than the stated value of the probability of transgression (0.05, 0.01, 0.001) (Horodnic S. A., 2008).

Table 5

ANOVA test results on the influence of culture media on *Phytophthora* species

Analyzed factors	*P-value*	
Phytophthora infestans colonies development on the four culture media	**0.54442**	NS – statistically insignificant
Number of spores of *Phytophthora infestans* pe ml on the four culture media	**0.95913**	NS – statistically insignificant
Phytophthora parasitica colonies development on the four culture media	**0.00191**	** - Distinct significant statistical influence
Number of spores of *Phytophthora parasitica* pe ml on the four culture media	**0.00202**	** - Distinct significant statistical influence

* - Very significant statistical influence (P-value < p = 0.001)

** - Distinct significant statistical influence (P-value < p = 0.01)

*** - Significant statistical influence (P-value < p = 0.05)

NS - Statistically insignificant (P-value > p = 0.05)

The P value for the *Phytophthora infestans* colonies development on the four culture media was 0.544426841 and the P value for the number of spores was 0.959134824, after processing the data we can say that all four culture media do not

influence statistically the development of *Phytophthora infestans* colonies or the number of spores pe ml, the P value for both factors being well above the maximum established limit of the probability of transgression (0.5).

From the ANOVA test applied to the data collected during the observations carried out in the case of *Phytophthora parasitica* fungus, it has been found that all four culture media, influence from a statistical point of view both the colonies development and the number of spores per ml concentration. Both values obtained ranged between 0.1 to 0.001 where it appears that the culture media have a distinct significant statistical influence.

CONCLUSIONS

The *Phytophthora infestans* de Bary colony. was best developed on Petri plates with PDA medium, where after calculating the average of nine plates, on the last day of observations a value of about 1.47 cm was recorded.

As with the colony development, the most favorable media for sporulation was the PDA media. After averaging nine plates, the maximum concentration of spores/ml was observed in four weeks, with a value of 2332000 spores/ml. A significant increase after the first four weeks, followed by decreasing the number of spores was observed on all of the sporulating culture media.

Phytophthora infestans de Bary. colonies developed on PDA and colonies developed on V8, we observed them as having an irregular shape, with a wavy edge and a curved profile. The colonies developed on a culture medium TA, have only very fine hyphae with a filamentous form and edge and a flat profile.

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Although the colony of *Phytophthora parasitica* Dast. developed with higher rate on V8 culture media, TA media ensures a more abundant sporulation at the end of the first four-week, the average of the nine culture plates showed a value of 2586000spores/ml. Very close to this value were the plates with V8 2554000 spores/ml., followed by the values recorded on PDA 2352000 spores/ml. and CMA with 1898000 spores /ml.

PDA developed colonies have a round shape with a whole edge with concentric zonality that is formed during the third week and an umbonate profile. CMA developed colonies had a filamentous form, with a full edge and a flat profile. Colonies developed on TA media had a round shape, filamentous edges and crater form profile.

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