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Onosma (Boraginaceae) pollen interactions with *Bombus terrestris* (buff-tailed bumblebee) species in Jordan

Interacciones del polen de *Onosma* (Boraginaceae) con la especie *Bombus terrestris* (abejorro común) en Jordania

S. Al-Qura'n
Mu'tah University, Department of Biology, Faculty of Science, Karak, Jordan.

Abstract

The relationships between pollen morphology of three *Onosma* species and their interactions with their insect visitors *Bombus terrestris* during springs of 2012 and 2013 were studied in Jordan. Pollen morphology characteristics (type and size of pollen grains, pollen shape, symmetrical structures between pollen grains and the pollen visitors, polar and equatorial measurements, P/E ratios, structures of endo- and ecto- apertures) were investgated to formulate any possible way of interactions. Certain kind of bees *Bombus terrestris* (buff-tailed bumblebee) was observed as the most abundant visitor especially on the flowers of *Onosma* in April although many other honey bees were observed like *Apis mellifera, Bombus impatiens, Apis dorsata* and *A. florea* but in very low frequencies. *Onosma* species in Jordan as all boraginacean species produces a large amount of whitish polliniferous dust, thus considered as a potential source for all pollen visitor species of bees, especially Bombus terrestris known as buff-tailed bumblebee. Three *Onosma* species were investigated palynologically by LM and SEM. The pollen class is either 3 zonocolporate (*Onosma aleppica* Boiss and Onosma frutescens Lam.) or 4-zonocolporate (*Onosma cinerea* Schreber). Polar measurements ranges are (37-43) µm for *Onosma aleppica* Boiss, (42-45) µm for *Onosma cinerea* Screber and (41-44) µm for *Onosma frutescens* Lam. Equatorial measurement ranges are (26-34) µm for *Onosma aleppica* Boiss, (28-34) µm for *Onosma cinerea* Screber and (28-33) µm for Onosma frutescens Lam. P/E ratio ranges between (1.41–1.39) for *Onosma aleppica* Boiss, (1.49-1.33) for *Onosma cinerea* Schreber and (1.45-1.28) for *Onosma frutescens* Lam. Pollen outline has been recognized, the equatorial view for all *Onosma* species examined was rectangular-elliptic; the polar view was triangular for all species. P/E ratio view was erect for all species examined. The most important diagonistic factor in recognition of pollen morphology of *Onosma* species is in the endoaperture and ectoaperture which showed some kind of variation reflected on their pollen visitor association. The morphology of ectoaperture structures (colpi, fastigium and margins) for the three species exhibited short colpi, absence of fastigium with indistinct margins for *Onosma aleppica* Boiss, long colpi, presence of fastigium and indistinct margin for *Onosma cinerea* Schreber and short colpi, presence of fastigium and distinct margin of *Onosma frutescens* Lam. The morphology of endoaperture structures (colpi, costae and margins) has reflected also distinguished variation; *Onosma aleppica* Boiss showed short colpi, presence of costae and indistinct margin, *Onosma cinerea* Schreber showed short to long colpi in general, absence of costae and distinct margins

Keywords: *Onosma*, Boraginaceae, pollen interactions, *Bombus terrestris,* Jordan

Resumen

Se estudiaron las relaciones entre la morfología del polen de tres especies de *Onosma* y sus interacciones con los insectos visitantes *Bombus terrestris* durante las primaveras de 2012 y 2013 en Jordania. Las características morfológicas del polen (tipo y tamaño de los granos de polen, forma del polen, estructuras simétricas entre los granos de polen y los visitantes del polen, medidas polares y ecuatoriales, relaciones P/E, estructuras de las endo y ecto aperturas) fueron investigadas para establecer cualquier posible modo de interacción. Cierto grupo de abejas *Bombus terrestris* (abejorro común) fueron observadas como las visitantes más abundantes en las flores de *Onosma* especialmente en abril, aunque fueron observadas muchas otras abejas de miel, tales como *Apis mellifera, Bombus impatiens, Apis dorsata* y *A. florea,* pero con muy poca frecuencia. Las especies de *Onosma* en Jordania, al igual que todas las especies de boragináceas, producen una gran cantidad de polvillo polinífero blanquecino, por lo tanto son consideradas como atractivo potencial de todas las especies de abejas visitantes del polen, especialmente *Bombus terrestris* conocida como abejorro común. Tres especies de *Onosma* fueron estudiadas palinológicamente con microscopio óptico y microscopio electrónico de barrido. El tipo de polen es 3-zonocolporado (*Onosma aleppica* Boiss y *Onosma frutescens* Lam.) y 4-zonocolporado (*Onosma cinerea* Schreber). Los rangos de las medidas polares son (37-43) µm para *Onosma aleppica* Boiss, (42-45) µm para *Onosma cinerea* Schreber y (41-44)

µm para *Onosma frutescens* Lam. Los rangos de las medidas ecuatoriales son (26-34) µm para *Onosma aleppica* Boiss, (28-34) µm para *Onosma cinerea* Schreber y (28-33) µm para *Onosma frutescens* Lam. Las relaciones P/E varían entre (1.41-1.39) para *Onosma aleppica* Boiss, (1.49-1.33) para *Onosma cinerea* Schreber y (1.45-1.28) para *Onosma frutescens* Lam. Los perfiles del polen han sido reconocidos. La vista ecuatorial para todas las especies de *Onosma* examinadas fue rectangular–elíptica; la vista polar fue triangular para todas las especies. La vista de la relación P/E fue levantada para todas las especies examinadas. El más importante factor de diagnóstico en el reconocimiento de la morfología del polen de las especies de *Onosma,* está en la endoapertura y la ectoapertura, las cuales mostraron cierto tipo de variación reflejada en su asociación con los visitantes del polen. La morfología de las estructuras de ectoapertura (colpos, fastigio y márgenes) exhibió colpos cortos, ausencia de fastigio con márgenes indistintos para *Onosma aleppica* Boiss; colpos largos, presencia de fastigio y margen indistinto para *Onosma cinerea* Schreber; y colpos cortos, presencia de fastigio y margen distinto para *Onosma frutescens* Lam. La morfología de las estructuras de endoapertura (colpos, costillas y márgenes) ha reflejado también variaciones distinguibles; *Onosma aleppica* Boiss mostró colpos cortos, presencia de costillas y margen indistinto; *Onosma cinerea* Schreber mostró colpos cortos y largos en general, ausencia de costillas y márgenes distintos.

Palabras clave: *Onosma*, Boraginaceae, interacciones del polen, *Bombus terrestris*, Jordania.

Introduction

Onosma as genus from Boraginaceae is well known and widely distributed in Jordan, which is located in unique position in the heart of the middle east between longitudes 53^0 40′ and 39^0 E, and between latitudes 29^0 30′ and 34^0 N, this location of Jordan gives this country extraordinary opportunity to comprise at least four main different phytogeographical elements from north to south and from west to east (mediteranean, irano-teranean, saharo-arabian and nubo-sudanian), surely this leaded to geobotanical variation which enriches the plant biodiversity in jordanian territories, and reflected over the high spectrum of plant species. *Onosma* is widely producing different kinds of pollineferous dusts especially during spring pollination times, which interconnected large variation of insects especially buff-tailed bomblebees as pollen visitors between the wild and cultivated species (Bender, 1974; Al-Quran, 1986; 1988; 2010; 2011).

Onosma genus has unique potential in production of a vast quantities of nectar-pollen complex secreted from a certain secretory cells mixed with white pollineferous material attracting different kinds of hymenopteran species. The investigation of pollen morphology of *Onosma* is considered a very important morphological evidence to investigate some aspects related to pollen class, endoaperture, ectoaperture structures, polar and equatorial measurements, P/E ratio and pollen outline shape and structure, to see whether some palynological variations among the examined species will exist, and will be relied on for such characterizations and differentiation among the *Onosma* species having the role for such attraction between the pollen grains and their visitors especially from *Bombus terrestris* species (Zohary & Feibrun-Dothan, 1962-1986; Adam, 2001; Khayyat & Mursi, 1981; Peter, 1994; Prance, 2001; Punt *et al*.,1994).

The floral parts of *Onosma* species are consisting of gametophytic male structures represented by androecium and gametophytic female structures represented by gynoecium located in the central part of the flowers surrounded externally by

perianth parts represented by calyx and corolla, this kind of floral arrangement is typically found as general morphological shape of this family enabling the flowers of *Onosma* to attract certain types of buff-tailed bomblebees (*Bombus terrestris*) during the pollination times. Also the inflorescence of *Onosma* is similar to other boraginacean, composed of many simple clusters of several flowers. The presence of colored lobulated external purple glandular trichomes and hairs in form of papillae near the base of floral parts especially corolla, is considered the source of nectar production release high quantities of nectar-pollen substrate to facilitate this kind of pollen visitors' attraction (Zohary, 1973; Anderson & Gensel 1976; Boulos,1979; Al-Quran, 2005).

All the palynological terms and definitions used in general in such studies are related to the structures seen by LM and ultrastructural parts of the pollen grains seen by SEM are focusing in the ectoaperture and endoaperture of pollen grains, these structures are considered as a complex structures especially which deals with colpi, costae, fastigia; these structures are vary from species to species. These structures which are similar to echinae and micro-echinae in form of spine-like projections giving the pollen exine an ornamental view may be investigated to see whether they can depend upon as an important distinguishing complimentary characters in delimitation among the species of the same genus or the subspecies of the same species (Rodriguez *et al.*, 1998; Al-Quran, 2010; 2011; Sharma, 1974; Samways, 1990).

It is obvious from the previous studies the lack of any pollen insect associations image between the pollen grains of *Onosma* and its pollen visitors of buff-tailed bumblebees, which was the most intrinsic factor to initiate this kind of investigation, and in addition to that, these species are producing certain kinds of pollen grains visited by a different types of insects representing different orders and classes. Absolutely the floral structures of *Onosma* species with their pollen grains are considered to have the potentiality to form this kinf of specialized intimate relationship with pollen visitors to reinforce this association with pollen and nectar as rewards for the visitors. doubtless there was a correlations representing similarities which was lead to some kind of superimposing between the anther structures as the source of pollen grains, corolla bases as the source of nectar and the pollen visitors attracted, so these pollen morphological characteristics and the pollen-nectar as rewards to pollen visitors forming what is similar to blooming time. All previous works are stressing on the seasonality followed by the different pollen visitors of insects as the ultimate factor of initiation reflecting different observed patterns of visitor distribution among the examines species of *Onosma* for the collection of pollen-nectar as potential rewards introduced by these plant flowers (Thorp, 1979; Stickel *et al*., 2000; Adailkan & Guathaman, 2001; Boulos & Al-Eisawi, 1977; Boulos & Lehham, 1978).

The formulation of pollen-insect association between the plant and the pollen morphology of *Onosma* species interactions with their insect visitors *Bombus terestris* (buff-tailed bomble bees) is surely reflecting some kind of specialized structural adaptations. Pollen morphology traits regarding ectoaperture, endoaperture morphology of pollen grains, fastigia, colpi of pollen grains, margins of polar and equatorial measurements, pollen type, size of pollen grains, pollen shape, symmetrical structures between pollen grains and the pollen visitors, and therefore P/E ratio

are playing a major rules to formulate any possible way of interactions. Buff-tailed bomble bees (*Bombus terrestris*) was the most observed and abundant especially on the flowers of *Onosma* in April.

Four main objectives of this study, they are: 1) to speculate whether *Onosma* plant flower having some kind of particular morphological and palynological characteristics to attract certain groups of pollen visitors over others. 2) to speculate whether the understanding of the flower rewards in form of nectar-pollen complex is considered as the motivating factor that may play the major role of such pollen visitor attractions. 3) to speculate whether the attractions between the pollen visitors with the pollen grains of flowering *Onosma* particularly in the field area have a clear investigated image concerning this kind of association.

Material and methods

The collected fresh polliniferous material from different sites representing the different jordanian territories, belonging to three *Onosma* species (*Onosma aleppica* Boiss, *Onosma cinerea* Schreber and *Onosma frutescens* Lam.) by using field collecting tools (field viales and small brushes). This material was acetolysed typically according to Erdtman (1960) standard method. So 25 randomly chosen acetolysed pollen grains from 5 different slides were prepared representing the pollen grains of *Onosma* species have been prepared to be studied to collect the data required in form of pollen measurements by LM. All palynological measurements related to the pollen morphological structures with their statistical calculations were estimated for the morphometric data. All the terminologies adopted were based on Punt *et al.* (1994). All LM micrographs and measurements were taken by using Nikon HFX-11 microscope using ocular micrometer scale by glycerin Jelly method.

For SEM studies, the treated pollen grains were coated by carbon layer first, then by gold layer to increase the conduction and electron yield rates, micrographs were taken by SEM.

All observations were taking place in the area of study of Ajlun in northern heights of Jordan. These observations were from April 2011 to April 2012 during the booming syndrome The methodology used for recording the species of *Onosma* as plant species visited by buff- tailed bomble bees as pollen visitors was according to visitors and pollen counts, photographic records and specimens collection with entomological nets.

This study of plant-insect interaction is occurred in the plant communities mainly dominated by certain types of boraginacean plants especially *Onosma* species and most of them are commonly known as entomophilous plant species. In each *Onosma* species studied and visited regularly by pollen visitors, It showed some kind of correlation between flowering phenology and insect censuses with buff-tailed bomble bees (*Bombus terrestris*), the pollen grains were collected and photographed by LM and SEM.

Results and discussion

All LM and SEM micrographs taken for the pollen grains of *Onosma* species studied were exposed with their full captions (Fig. 1, 2 and 3). All palynological data concerning the observations and measurements of the pollen grains of the studied *Onosma* species were constructed (table 1 and 2). The data are collected in two main categories (1) measurements concerning

the pollen morphology of the examined *Onosma* pollen grains (2) All observations collected from the field related to pollen-insect interactions concerning the pollen of *Onosma* and buff-tailed bomble bees as pollen visitors. Related to the first category; the pollen class of the examined *Onosma* species is either 3- zonocolporate (*Onosma aleppica* Boiss and *Onosma frutescens* Lam.) or 4-zonocolporate (*Onosma cinerea* Schreber). Polar measurements ranges are identified, they were as follows: (37-43) µm for *Onosma aleppica* Boiss, (42-45) µm for *Onosma cinerea* Screber and (41-44) µm for *Onosma frutescens* Lam. Equatorial measurement ranges are also identified as follows: (26-34) µm for *Onosma aleppica* Boiss, (28-34) µm for *Onosma cinerea* Screber and (28-33) µm for *Onosma frutescens* Lam. P/E ratio are found to be ranged between (1.41–1.39) for *Onosma aleppica* Boiss, (1.49-1.33) for *Onosma cinerea* Schreber and (1.45-1.28) for *Onosma frutescens* Lam. The morphology of ectoaperture structures (colpi, fastigium and margins) for the three species exhibited short colpi, absence of fastigium with indistinct margins for *Onosma aleppica* Boiss, long colpi, presence of fastigium and indistinct margin for *Onosma cinerea* Schreber and short colpi, presence of fastigium and distinct margin of *Onosma frutescens* Lam. The morphology of endoaperture structures (colpi, costae and margins) has reflected also distinguished variation; *Onosma aleppica* Boiss showed short colpi, presence of costae and indistinct margin, *Onosma cinerea* Schreber showed short to long colpi in general, absence of costae and distinct margins. Pollen outline has been recognized, the equatorial view for all *Onosma* species examined was rectangular-elliptic; the polar view was triangular for all species. P/E ratio view was erect for all species examined. The most important diagonistic factor in recognition of pollen morphology of *Onosma* species is in the endoaperture and ectoaperture which showed some kind of variation reflected on their pollen visitor association. The morphology of ectoaperture structures (colpi, fastigium and margins) for the three species exhibited short colpi, absence of fastigium with indistinct margins for *Onosma aleppica* Boiss, long colpi, presence of fastigium and indistinct margin for *Onosma cinerea* Schreber and short colpi, presence of fastigium and distinct margin of *Onosma frutescens* Lam. The morphology of endoaperture structures (colpi, costae and margins) has reflected also distinguished variation; *Onosma aleppica* Boiss showed short colpi, presence of costae and indistinct margin, *Onosma cinerea* Schreber showed short to long colpi in general, absence of costae and distinct margins.

With respect to the second category; the insect-plant initiation of interactions is refered to the pollen morphology of studied species (long or short colpi, distinct or indistinct endoapertures and ectoapertures, presence or absence of fastigia and costae). Also *Onosma* species in Jordan as well as for all boraginacean pollen grains produces a large amount of whitish polliniferous dust and constitutes a potential source of pollen for different species of buff-tailed bomble bees, providing an interesting field for attraction of such insects. It is worthy to say that depending on the previous palynological results and measurements obtained from pollen grains of *Onosma* studied, is obviously cannot be relied on in delimitations of *Onosma* species at species level, but surely can be relied on to explain the intimate relationship between the morphology of pollen grains with pollen visitors of *Bombus terrestris*, to explain this point clearly, it is very important to reconize

the presence of such differences between the pollen grains related to polar (P) and equatorial measurements of pollen grains exhibited by an ecto-and endo-apertures morphology which are playing the role of variations in pollen-visitor attraction among the different species of *Onosma*, and this was seen clearly in most of the pollen-insect communities studied showed the presence of specialized and intimate insect-flower associations, this refers in some aspects to the complicated *Onosma* flower morphologies which hinders or facilitates this kind of visitation depending on the species visited by wide range of other insect groups. So it is clear that *Bombus terrestris* colonies are active approximately in all over the year seasons extending from winter to summer to late autumn to collect as large possible quantities of nectar as they can , so these foragers appeared in the study area early in the season on plants that show high densities and high nectar productions especially on *Onosma* flowers.

In more details, the investigated pollen grains of *Onosma* were indicating the presence or absence of some morphological characters especially in the pollen ornamentations, the size of the pollen grains and the occurrence of the little complex variated of some pollen ecto-and endo-apertures of some species of *Onosma* is highly related to the shedding process of these grains inside of the poricidal anthers during anthesis process. The shedding process is playing the most important role in playing the execution through pollen visitor vibrations or "buzz pollination" by visiting buff-tailed bomble bees. Exactly by relying on the same principle of expression, wherever the larger the pollen grains of some studied *Onosma* species with high complex pollen endoaperture and ectoaperture ornamentations, wherever the case to form deposits blocking the anther anthesis to expel the pollen grains is rising. So in this way, it is worthy to assure the presence of close relationship between the type and size of pollen morphology and the pollination syndrome by "buzz pollination" reinforcing the observations collected from the fields. Easily also, it is obvious that small sized pollen grains with little ornamentation will be expelled more easily from the poricidal anthers during the vibration of the honey bees (Erickson,1975; Edmond, 1984; Adam, 2001; Al-Quran, 2004a, 2004b; Buchman, 1986; Thorp, 1979).

The observations and data collected showed the presence of other types of insects as honey bees (*Apis mellifera*), *Bombus impatiens, Apis dorsata* and *A. florea* and wasps from (*Pterygophorus insignis*) species but in low frequencies. All the pollen visitors observed used the "buzz pollination" procedure during their foraging behaviour. The previous published studies report that "buzz pollination syndrome" requires hymenopteran species with a specific behavior for pollen removal which is typically applied to the bees from *Apis mellifera* and *Bombus terrestris* species. It is important to demonstrate the importance of this kind of association between the partners to improve the apiculture management in honey. So the previous studies were trying to provide evidences of such pollen collection strategy from pollen grains of *Onosma* species by such kind of bees (Buchman, 1986; Adam, 2001). There was a lot of previous workings investigating similar aspects concerning the interrelationship working with *Vespa orientalis* L. on the pollen grains of *Anchusa* species. These results obtained from the previous workings approved these results obtained since they showed that the collection of pollen grains by the pollen

visitors due to such pollen characteristics related to the ornamentations of pollen endo-and ecto-apertures, and these results consequently are clerifying the importance of the palynological data to understand this association depending on pollen morphology of pollen grains and the visitors (Pyle *et al*., 1991; Rates, 2001; Peter, 1994; Ricklefs, 2004; Anderson & Gensel, 1976; Friedman *et al*., 1986; Joud *et al*., 2001; Eddouks *et al*., 2002).

Edmonds (1984) obtained from similar studies on some Solanaceae species (*Solanum*) species showing that the morphological variations in pollen grains of the genus *Solanum* (Solanaceae) reflected no any significant morphological variations, because theyn refered only to exine ornamentation level related to the certain structures of ecto-and endo-apertures morphology, similar to what is happening in *Anchusa* species.

The variation in ornamentation character of ecto-and endo-apertures morphology of pollen grains is considered the crucial factor determining the phylogenetic relationships among the species in addition to genomic combinations, isolation and speciation, this result confirmed by Al-Quran (2004) through his study of pollen grains of 11 species of *Hyoscyamus* collected from different parts of Jordan.

Other researches (Buchmann, 1986; Erickson, 1975; Thorp, 1979) were going further by demonstration the presence of certain electrostatic forces, facilitating the attachment of the pollen to the body of the insect at the moment of pollination by vibration through the blooming syndrome, which facilitates finally its transference to the stigma. So the presence of a cost-benefit relationships an important fact involved in the pollination syndrome by vibrations of the bees is the major aspect. Of course the bees are exerting a large amount of energy to move their flight muscles and to complete the vibration process. Buchman (1986) confirmed the presenceto a certain degree the presence of waste of pollen grains in this process, and only pollen with a high protein level could justify this plant-insect relationship.

Conclusion

The building of such specialized and intimate associations between the pollen morphology of *Onosma* and the pollen visitor (*Bombus terrestris*) is clearly due to the need of such bees to collect the nectar and pollen by default, this certainly preclude a certain morphological adaptational similarities between he partners in the plant-pollinator interactions of the community to collect a high quantities of nectar forage as soon as possible in short time and straight way. These results may give an indication that flowers with similar reward composition tended to attract similar groups of visitors. In this regard, the pollen visitor responses to flower nectar as floral reward and considered as main motivating factor rather than responsing to the flower morphology.

The using of the palynological study as palynological evidence in taxonomy and delimitation of the three *Onosma* species is mainly not worthful, because the depending on the pollen structures and measurements seen by LM and SEM can't be depend upon in such kind of delimitation because of the high degree of similarities between the examined species which showed no clear differences in the diagonistic features exhibited, consequently they have exhibited the same inter-relationship with hymenopteran species. By the same reason, it is worthy to depend on the morphological variations of pollen grains to explain why

do some pollen visitors are attracted to certain plant pollens more than the others

The previous results obtained by the researcher have showed the presence of four different aspects must be taken in consideration to reach a better understanding the linkage between pollen morphology of *Onosma* and the pollen visitor (*Bombus terrestris*): (1) phenology construction between the the partners associated (2) morphology of the pollen visitors, (2) pollen morphology, (3) nectar-pollen rewards given by the flower to the the pollen visitor. In other words, the better understanding of importance between pollen morphology, floral rewards and flowering time of each plant species is very important to to interpreting why do certain insects to be attracted to a certain flowers definitely not to others.

This kind of studies with other complementary studies should be intensified further to have a better understanding of the existing relationships between the pollinators and the attracted species to justify the occurrence of pollination syndrome by vibration found in the observed bees in form of "buzz pollination", so this kind of association is notonly foraging strategy, but in rather is intimate reciprocal kind of exchange benefits between the partners.

Finally the main conclusion can be relied on is, wherever the pollen grain has a reduced size, the ornamentation aspects in ecto-and endo-apertures and psilated exine all together sharing to give the outline of pollen moprphology to appropriate certain behavioral form, allowing pollen grain access by small sized bees as observed with buff-tailed bomble bees species.

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Anexo

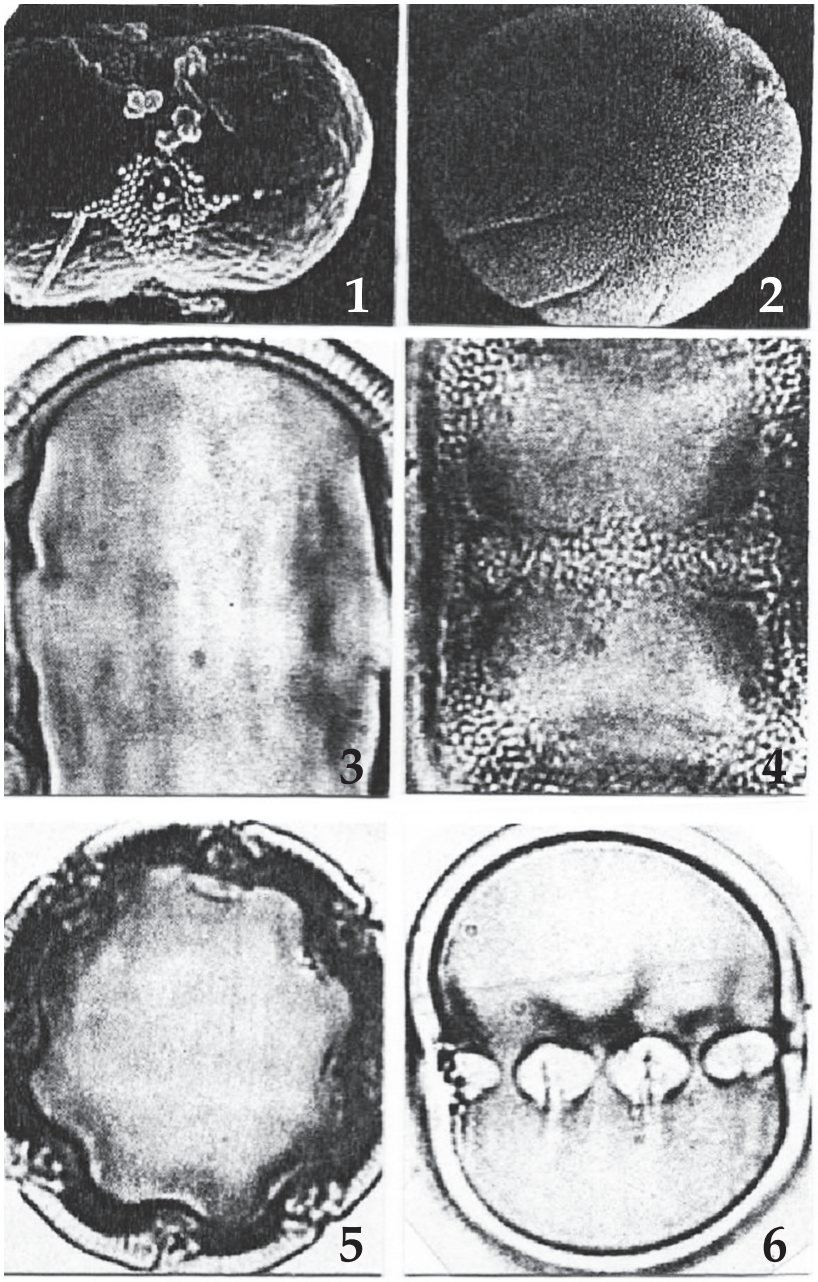

Fig . 1

1. SEM micrograph (1800 X), showing pollen grains of Anchusa aegyptiaca (L.) DC., polar view.
2. SEM micrograph (1900 X), showing pollen grains of Anchusa aegyptiaca (L.) DC., polar view.
3. LM micrograph (1000 X), showing pollen grains of Anchusa itaica Retz., equatorial view.
4. LM micrograph (1100X), showing pollen grains of Anchusa strigosa Banks & Sol., equatorial view.
5. LM micrograph (1200X), showing pollen grains of Anchusa itaica Retz., polar view.
6. LM micrograph (1160X), showing pollen grains of Anchusa strigosa Banks & Sol., equatorial view.

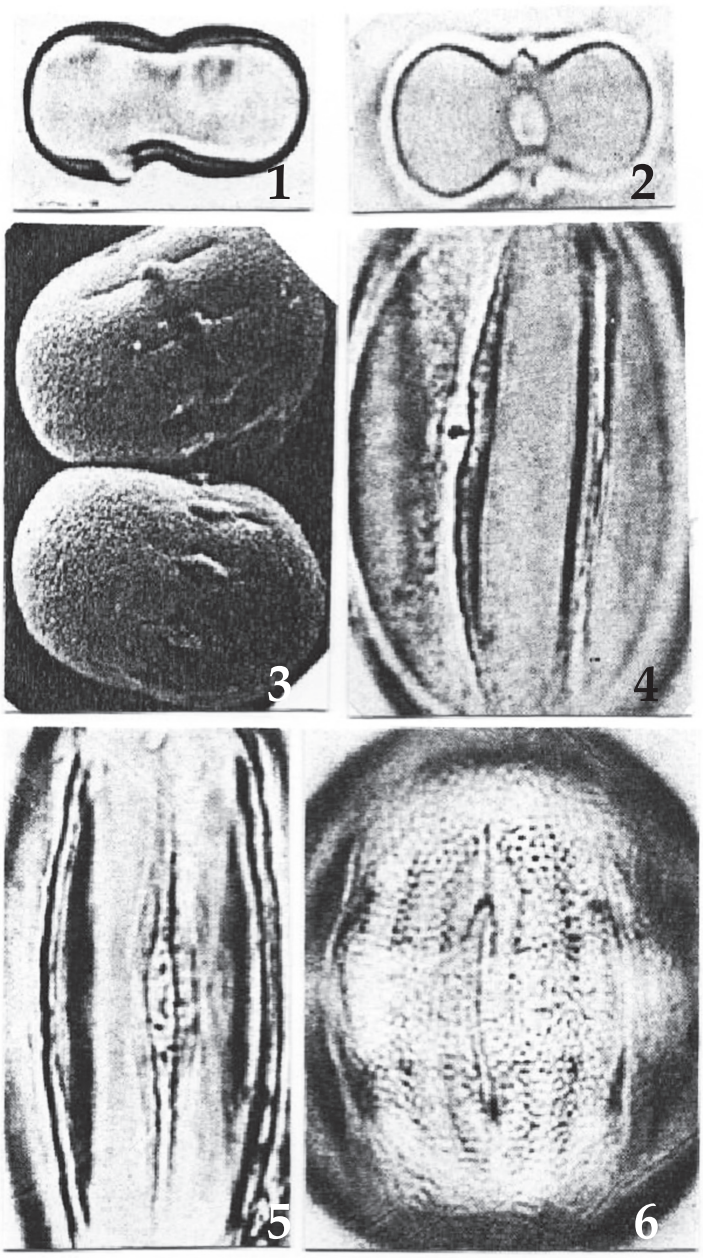

Fig . 2

1. LM micrograph (1100 X), showing pollen grains of Anchusa aegyptiaca (L.) DC polar view.
2. LM micrograph (1200 X), showing pollen grains of Anchusa itaica Retz., polar view .
3. SEM micrograph (1700 X), showing pollen grains of Anchusa strigosa Banks & Sol., equatorial view .
4. LM micrograph (1100 X), showing pollen grains of Anchusa aegyptiaca (L.) DC, equatorial view .
5. LM micrograph (1000 X), showing pollen grains of Anchusa itaica Retz., ornamentation in focus.
6. LM micrograph (1100X), showing pollen grains of Anchusa aegyptiaca (L.) DC, ornamentation in focus.

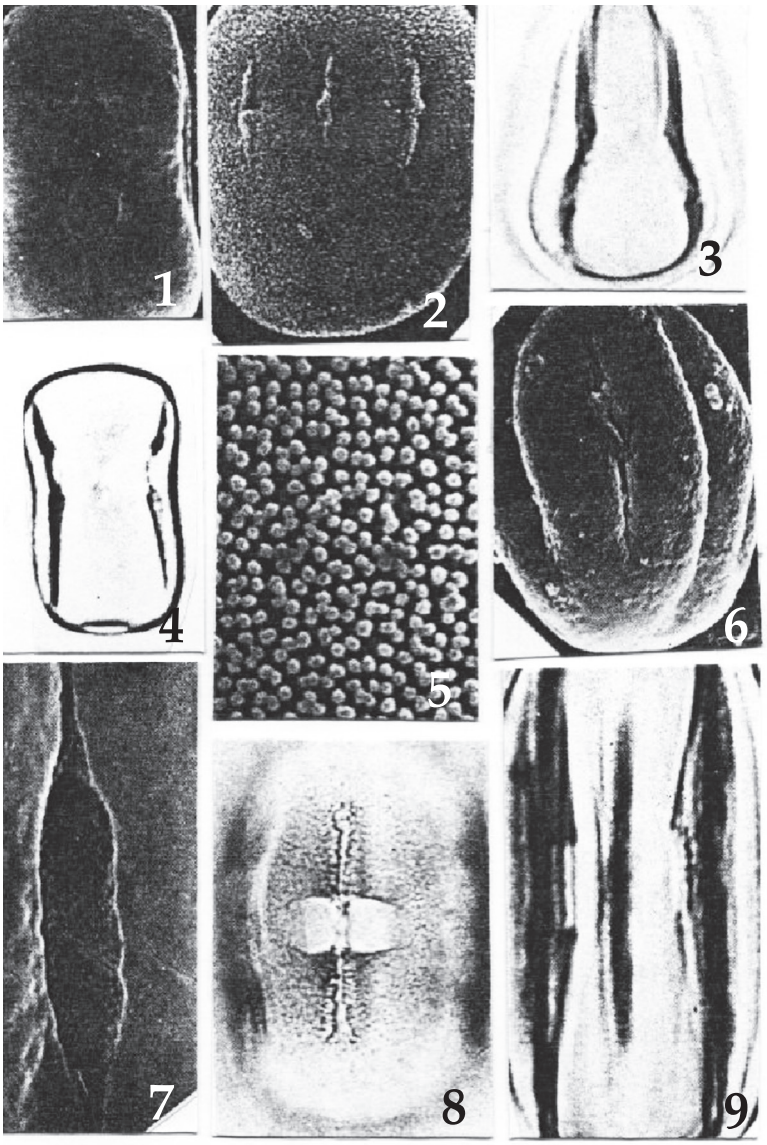

Fig . 3

1. SEM micrograph (1900 X), showing pollen grains of Anchusa itaica Retz., polar view.
2. SEM micrograph (1900 X), showing pollen grains of Anchusa itaica Retz., with ornamentation.
3. LM micrograph (1100 X), showing pollen grains of Anchusa itaica Retz, equatorial view.
4. LM micrograph (1000 X), showing pollen grains of Anchusa aegyptiaca (L.) DC, 3 –Zonocolporate.
5. SEM micrograph (2220 X) , showing pollen grains of Hyoscyamus reticulatus L., endoaperture colpi.
6. SEM micrograph (2500 X), showing pollen grains of Anchusa aegyptiaca (L.) DC, 3-Zonocolporate with colpi ends.
7. SEM micrograph (1250 X), showing pollen grains of Anchusa itaica Retz., equatorial view.
8. LM micrograph (1030X), showing pollen grains of Anchusa aegyptiaca (L.) DC., equatorial view.
9. LM micrograph (1100X), showing pollen grains of polar view

Table 1. List of plant species examined with relative to their pollen class, measurements of polar (P) and equatorial (E) views in µm, and P/E ratio.

No.	Species	Pollen class	P (µm)	E (µm)	P/E
1	*Onosma aleppica* Boiss	3 - Zonocolporate	37 - 43	26 - 34	1.41 – 1.39
2	*Onosma cinerea* Schreber	4 – Zonocolporate	42 - 45	28 - 34	1.49 – 1.33
3	*Onosma frutescens* Lam.	3 – Zonocolporate	41 – 44	28 - 33	1.45 – 1.28

Table 2. List of plant species examined relative to their outline views, ectoaperture and endoaperture structures

No.	Species	Outline			Ectoaperture			Endoaperture		
		E view	**P view**	**P/E view**	**colpi**	**fastigiu**	**margin**	**colpi**	**costae**	**margin**
1	*Onosma aleppica* Boiss	rectangular-elliptic	triangular	Erect	s	-	ind	s	+	ind.
2	*Onosma cinerea* Schreber	elliptic	triangular	Erect	l	+	ind.	s-l	-	ind
3	*Onosma frutescens* Lam.	elliptic	triangular	Erect	s	+	d.	s	+	d.

P : polar **E : equatorial**

+ : present **- : abscent**

ind : indistinct **d : distinct**

s : short **l : long**