FORUM

The Uses of Pollen and its Implication for Entomology

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Os Usos do Pólen e suas Implicações na Entomologia

RESUMO - Palinologia é o estudo dos grãos de pólen produzidos por plantas sementeiras (angiospermas e gimnospermas) e esporos (pteridófitas, briófitas, algas e fungos). O pólen representa a flora terrestre e pode ser útil em muitas disciplinas: é único, não deteriora facilmente e é um marcador natural. O pólen pode ser usado para determinar os mecanismos de polinização, os recursos de forrageamento, as rotas de migração e locais fontes de insetos e outros polinizadores. O pólen fornece meios para análises paleoambientais de rochas terrestres sendo de interesse de geólogos. Também auxilia a explicar as correlações de ambientes terrestres com as sucessões marítimas e é usado para determinar trocas ecológicas e ambientais. Recentemente, a palinologia tem sido utilizada nos meios forenses. Esse artigo introduz a idéia do uso do pólen em várias disciplinas e, em particular, na entomologia.

PALAVRAS-CHAVE: Pólen, entomopalinologia, forense, aerobiologia.

ABSTRACT - Palynology is the study of pollen grains produced by seed plants (angiosperms and gymnosperms) and spores (pteridophytes, bryophytes, algae and fungi). It represents the land flora and can be use in many different disciplines. Pollen is distinctive, does not easily decay and is a natural marker. Pollen can be used to determine pollination mechanisms, foraging resources, migration routes and source zones of insects and other pollinators. It provides a means for paleoenvironmental analyses of terrestrial rocks and is of interest to geologists. Pollen also aids in the correlation of terrestrial with marine successions and is used to determine environmental and ecological changes. Recently, palynology is used in forensics. This manuscript introduces the ideas of using pollen in a variety of disciplines, in particular in entomology.

KEY WORDS: Pollen, entomopalynology, forensics, aerobiology.

Palynology is the study of pollen grains produced by seed plants (angiosperms and gymnosperms) and spores (pteridophytes, bryophytes, algae and fungi). Pollen and spores differ in their function, but both result from cell division involving a reduction by half of the chromosome content (meiosis) (Moore *et al.* 1991). Pollen grains house the male gametes, but spores are usually the resting or dispersal phase of the fern, algae, etc. Because of the larger size and the importance of pollen in pollination and to insects, pollen will be emphasized.

Pollen grains vary in shape from spherical to elliptic to triangular. Most pollen grains range from about 4 to 250 ìm. Pollen grains often have openings (pores) or furrows (colpus = singular, colpi = plural). A pollen grain with one colpus is called monocolpate. A pollen grain with three pores is triporate and one with three pores within three colpi is termed tricolporate. The outside layer of the pollen grain can be smooth (psilate), net-like (reticulate), or looks like a ball of string (striate). Some pollen grains even have spine like projections (echinate).

It is common knowledge that pollen is a major cause of allergies. However, pollen can be used to determine insect migration, insect food sources, honey types and in forensics, climatic changes, etc. There are several reasons pollen is used in these studies. First, pollen grains are distinctive, easily recognizable and identifiable to the family, genus and often species rank. Thus, very specific information can be obtained. Second, pollen is made up of sporopollenin that is durable and does not easily decay. Third, from the identification of the pollen, the geographical origin of the plant from which the pollen came can be determined.

The word pollen comes from a Latin derivative meaning fine flour or dust (Jarzen & Nichols 1996). Grammatically, the word pollen is a collective noun and is always treated as a singular noun, although it refers to many individuals. A single individual is called a pollen grain.

The production of pollen is a complex process that differs between the cone bearing and flowering plants. Stanley & Linskens (1974) and Shivanna & Johri (1989) describe at length the development and differentiation of pollen, including the biochemical, cytological and physiological aspects. One thing that all seed plants have in common is that the pollen must be transferred from the male structures (anthers) to the female structures (stigma). This transference of the male to the female is called pollination.

The diversity of floral morphology displayed by the flowering plants is incredible. Flowering plants are adapted to enhance successful pollination and fertilization under widely varying conditions (Jarzen & Nichols 1996). Numerous and often bizarre methods have developed to ensure that pollination occurs. Some pollination methods use pollinators such as insects (entomophily), bats (chiropterophily), birds (ornithopily), reptiles (saurophily) and small non-flying mammals (therophily) all transfer pollen from the male to the female parts of the plant.

Hummingbirds often land on the petals of the claret cup cactus (*Echinocereus coccineus* G. Engelmann). While holding on tightly to the petals, they will dip their entire body into the flower and sip from the nectaries at the base of the stigma. As they are doing so, they get dusted on the forehead with pollen by the anthers.

In Australia, a species of *Banksia* (Proteaceae) is pollinated by native rats (Carpenter 1978). A species of lizard has been observed visiting and drinking the nectar of *Aloë* (Elvers 1977). This study indicates the importance of reptilian pollinators in the past for pollinating seed-bearing plants. Reptile coprolites (fossilized feces) recovered from Jurassic sediments indicated that these animals browsed on the pollen-bearing plant structures (Harris 1945, 1956). It has been suggested that reptiles, not beetles, were the original pollinators of angiosperms (Hughes 1976). The most sophisticated pollination mechanisms have been evolved in the orchid family (Orchidaceae). Some bee orchids are pollinated by male wasps because the orchid flowers evolved to mimic female wasps.

Wind pollination (anemophily) is a random phenomenon because the pollen is carried by wind currents and may or may not reach the female parts of the same species. Anemophily is considered a primitive pollination method (Crane 1986) and prevails among the modern gymnosperms (cone-bearing plants) with the exception of some cycads and Gnetales (Faegri & van der Pijl 1979). Some flowering plant families (Poaceae, Cyperaceae, etc.) have adapted to being almost exclusively wind pollinated. Even some species of entomophilous plant families are wind pollinated (Jarzen & Nichols 1996). A common error is to take species as obligate anemophilous or entomophilous. A better designation for taxa exhibiting these evolutionary mechanisms would be facultative. In other words, just because a plant has been historically considered anemophilous does not indicate that insects and other animals do not pollinate that species and do not visit the flowers for pollen and or nectarBlack willow, *Salix nigra* H. Marshall, is a good example of an anemophilous species that is regularly visited by honeybees and other insects for pollen and nectar.

Pollen can be transported further by water and eventually can become incorporated into the lake or ocean sediment and become fossilized. When pollen is produced by plants growing near ponds or small lakes it may be directly incorporated into the sediments of the water body and will therefore reflect the kinds of plants growing in close proximity. Pollen from trees will be transported to the basin by wind and/or water. This pollen reflects the regional vegetation (Janssen 1984). Pollen derived from local sources provides information useful in paleoecological interpretation and pollen from regional origins is more useful in biostratigraphic studies (Jarzen & Nichols 1996).

Pollen represents terrestrial flora and can be of use in many disciplines. It can be used to determine pollination mechanisms, foraging resources, migration routes and source zones of insects and other pollinators. Pollen provides a means for paleoenvironmental analyses from terrestrial rocks and is of interest to geologists. It also can aid in the correlation of terrestrial with marine successions and to determine environmental and ecological changes.

Entomopalynology

Entomopalynology is the study of pollen that is associated with insects. Although it is a relatively new term, the study of the association of insects and pollen is not. This field of palynology includes many diverse disciplines including research on honeybees, melissopalynology, foraging distances, etc., but also pollination biology and migration patterns.

Flowers and insects co-evolved. The role of insects in pollinating flowers is common knowledge. Data obtained in pollination biology are useful for many reasons. First, the yield of many crops such as apples, almonds, peaches, melons, etc. are dependant on the pollinators that visit their flowers. When there are abundant pollinators, yields are usually increased. Second, some pollinators are also pests on crops, in orchards, etc. The more knowledge about these pests, the better and more efficient management practices can be developed. Third, the habit, habitat and live cycles of many pollinators are unknown. How often do various pollinators visit flowers? How many flowers are visited? What time of the day are the flowers visited? Are the pollinators after nectar, pollen, or both? How does genetically modified plants affect the pollinators? What would happen to the plant species if the pollinators were extirpated? Without knowing more about flowers and their pollinators, many of these questions cannot be answered.

Adults of numerous insect species feed on nectar, pollen and other plant exudates that are frequently associated with flowers. As a result of this feeding activity, these adults become contaminated with pollen. The identification of this pollen can be used to determine the insect's foraging resources, the migration patterns and the origin. Foraging resources are important to beekeepers for maintaining healthy beehives. Many homeowners specifically grow certain plant species to attract various insects like butterflies.

Migration routes and patterns are important because beneficial insects like the monarch butterfly migrate during particular months and along predictable corridors to a specific area in Mexico each winter. From this pattern, routes and wintering areas can be protected to ensure the survival of the species. Unfortunately, not all insects are beneficial in an economic sense. Although the adult insect may help the plants by pollinating them, the larvae of that insect may destroy other plants.

There is no universal flower type that is visited by all pollinators. It is the variations of the floral type that reflects the adaptation of the flowers to pollination by restricted groups of pollinators. Flowers that have nectaries are usually visited for both the nectar and pollen but flowers without nectaries are visited only for the pollen.

Most plants show daily peaks of nectar secretion and often of sugar concentration. In the middle of the day most flowers have maximum secretion indicating that this peak is an adaptation to the time of pollinator activity (Proctor & Yeo 1972). Stress on the plants can reduce the amount of nectar produced during mid-day. Nectar concentration may increase by evaporation or decrease by dilution caused by rain (Jaeger 1957). Pollen can fall into the nectar due to the wind or disturbance by pollinators. Once in the nectar, it can become attached to a pollinator when the pollinator is feeding on nectar.

The color and texture of the flower are important for attracting pollinators. Glossy, matt, or velvety textures of the petals often increase the intensity and the brightness of the petal color. Often petals may be velvety on the outside, but glossy towards the inside.

Another important aspect of the flower is a platform on which insects or other pollinators can land. The platform is usually the petals or the stigma itself that is adapted for this function. Anemophilous flowers are usually pendulous and flexible and therefore, are more difficult for a pollinator to land on.

Coleoptera. Although beetles are the largest order of insects, they have been often overlooked as pollinators because the pollination process (Cantharophily) is relatively unexciting due to the lack of specialization (Faegri & van der Pijl 1979). Cantharophilous flowers are usually large, single, dull in texture, greenish or off-white in color and heavily scented. Scents include the spicy scent of many crab apples (*Malus* spp.) to the odor of decaying organic material. Most beetle-pollinated flowers are flattened or dish shaped, with pollen easily accessible. Flowers adapted for beetle pollination have the seeds well protected from the jaws of the beetles (Grant 1950). Traps force the beetles to stay in the flower longer. The aquatic aroid has a 30 cm long underwater tube that traps the beetles and keeps them overnight underwater (Corner 1964).

Beetles seem to be more important as pollinators in semi-desert regions like South Africa and California (Grant 1950). In many tropical beetle-pollinated flowers, both the beetle and flower have retained their primitive characters. In fact, many of the primitive beetle blossoms look like beetles. Sometimes the plants use mimicry and strong odors lure beetles to the flower. Flowering plants that are beetle pollinated include *Nymphaea*, *Sambucus*, *Magnolia*, *Degeneria*, some species of *Rosa* and some species of the family Apiaceae (Faegri & van der Pijl 1979, Heiser 1962). Pollen from these taxa ranges from the large monocolpate grains of *Magnolia* (70 ìm in diameter) to tricolporate medium size grains in members of the Apiaceae. The ornamentation of many of these grains is scabrate to reticulate.

Pollen from a large diversity of plant species has been found in association with the insect pests such as boll weevils (*Anthonomus grandis* Boheman) and Mexican corn rootworms (*Diabrotica virgifera zeae* Krysan & Smith) (Hardee *et al.* 1999; Jones & Coppedge 1999, 2000; Jones *et al.* 1993). How much actual pollination occurs from these insects is unknown.

Diptera. The greatest variation of pollination methods is found among the plants that are fly pollinated (Faegri & van der Pijl 1979). Many of the flies that feed on exposed fluids also eat small solid particles including pollen grains. One of the first palynological studies of pollinating insects examined the stomach contents of some flies that fed on nectar and pollen (Proctor & Yeo 1972). Flies are important pollinators under certain climatic conditions because they are present at all times of the year. Some plants flowering at odd times of the year may be completely dependent on flies for pollination (Hagerup 1951, Kevan 1972). There are two types of fly pollination, myophily and sapromyophily.

In general, typical fly pollinated (myophily) flowers do not bloom regularly and are simple with very little depth. Flower color is usually pale with a dull texture. Nectar guides often occur. Nectar is open or easily available and the male and female parts of the flower are well exposed. Many of these flowers are scented, but for the most part, the odor is imperceptible. Plants that are fly pollinated include: *Euphorbia*, *Potentilla*, *Trifolium*, *Tradescantia* (pers. obs.), *Sedum* and various members of the Apiaceae, Brassicaceae and Orchidaceae families (Hagerup 1951). Pollen from these taxa ranges from small in the Brassicaceae (about 20 ìm) to the entire pollen producing structure, the pollinia of Orchidaceae. Ornamentation varies from psilate to reticulate.

Sapromyophily is fly pollination by carrion and dung-flies. The basis for sapromyophily is mimicry because the substances released by the flower activate the insect's instincts for feeding or oviposition (Meeuse 1966). Typical carrion or dung-flies are uninterested in the flowers as such, but go to the flower "expecting" to find rotting protein. Not finding the rotting protein, they will leave; therefore, most sapromyphilous flowers have traps to prevent the flies from rapidly leaving. The traps are similar to those found in the insectivorous plants and can be one-way bristles, slipways, or seesaw petals.

Characteristics of sapromyophilic flowers include: radial in shape, often with great depth, or lantern shaped, frequently with window openings through which the flies crawl into the blossom (or trap). Flowers have a dull texture and are dark colors of brown, purple and greenish. There are no nectar guides on the petals but often they are maculated (checkered with dark spots). Reproductive organs are generally hidden. Pollen grains are similar to those of myophilous taxa.

Female owl midges (Psychodidae) are especially attracted to certain species of *Arum* (Araceae) due to the odor of the flower. Mosquitoes utilize the protein in pollen grains that they split open (Downes 1974). Mosquitoes also pollinate *Habaneria* species (Orchidaceae) (Dexter 1913; Thien 1969a, b). Members of the genus *Bombylius*, sub-order Brachycera, are highly developed nectar feeders (Beattie 1972). While feeding on large tubular flowers or flowers with exposed nectar, *Bombylius* will hover or hold onto the flower with its legs and keep its wings constantly in motion (buzz pollination) (Simes 1946). Moving rapidly from flower to flower, they resemble small bumblebees.

The hoverflies, Syrphidae sub-order Cyclorrhapha, like *Bombylius*, hover in the air as they feed on flowers. Often brightly colored they are known as pollen eaters and visit tubular or convex shaped flowers (Baker 1957). Adult flies feed on pollen and nectar and the females require the amino acids in the pollen for the maturation of their reproductive system (Wratten *et al.* 1995). Hoverflies are potentially important in agricultural and horticultural crops as a biological control agent (Wratten *et al.* 1995). Information about the phenology and ecology of these species is limited and is needed to enhance biocontrol measures (Dean 1982, Chambers *et al.* 1983).

Lepidoptera. There are two groups of Lepidoptera, the butterflies and the moths. Butterflies are diurnal, while most moths are nocturnal. With a few exceptions, the only food sources for adults are liquids, mainly nectars. Lepidoptera are one of the best-known and most visible insect. Pollen is often involuntarily stuck to the proboscis or on body parts of the insect (legs, antennae, head, etc).

Typical flowers pollinated by butterflies (psychophily) are generally open during the day and closed at night, have a light aroma and are vividly colored including pure red. The flower is erect so that the butterfly can alight on the flower. The flowers have simple nectar guides with the nectaries usually hidden in narrow tubes or spurs. Flowers visited by butterflies include: *Silene, Rubus*, *Solidago, Salix, Lantana, Buddleia, Aster and Lonicera*.

Moth pollinated flowers (phalaenophily) are open at night and generally closed during the day, have a heavy sweet odor at night, are usually white or faintly colored pale rose or pale yellow. In low light conditions, these colors are more visible than other colors. The flowers are horizontal or pendent usually zygomorphic so that the moth may hover in front of the blossom without alighting. The petals are deeply dissected into lobes or they are fringed. Flowers generally lack nectar guides and the nectaries are deeply hidden in long tubes or spurs. Moth pollinated flowers usually produce more nectar than butterfly pollinated flowers. Moth pollinated plants include: *Gaura*, *Yucca, Lilium* and night blooming cactii. Pollen grains for Lepidoptera pollinated species vary from very large triporate grains in *Gaura* (160 ìm) to small tricolporate grains in *Salix* (20 ìm). Ornamentation of the pollen grains vary from echinate, to reticulate, to striate.

Hemiptera. This order of insects includes the aphids, leafhoppers and bugs. All have piercing and sucking mouthparts that are used for sucking the juices of plants or animals. However, aphids occasionally pollinate flowers and even the thrips are known to pollinate several different taxa (Hagerup 1950). Several families also visit flowers regularly. Research is being conducted to determine the importance of ollen in the diet of these insects.

Hymenoptera. The Hymenoptera comprise some of the most important pollinating insects. There are three major groups: wasps, ants and bees. Flowers utilized by these insects are flat with open nectaries. Some wasps (*Polistes*) are pollinators and even store nectar. Their ability to utilize flowers approaches that of the bees. Wasps are involved in a number of unusual pollination mechanisms; *Ophrys, Ficus*, etc. Wasp flowers have a dull texture and are usually in shades of browns: for example, *Scrophularia* (Shaw 1962).

Ants are notoriously fond of sugar and will use any source they can get from flowers to aphids to the sugar bowl. During brood rearing, they also need protein, which they often get from pollen. They are so small and their bodies ill adapted for pollen transport, that they enter in and out of flowers for protein or nectar without pollinating the flower. They are the ultimate nectar thieves. With better understanding of the ecology of ants, Vello & Magalhães (1971) report that the presence of the ant (*Aztecca*) increased the pollination of cocoa plants in Brasil.

Bees are better adapted for pollination than any other group of insects. They range from the simple solitary bee to the complex social bee. In the tropics, carpenter bees (*Xylocopa*) are important pollinators even though they have a tendency to steal nectar. Among the solitary bees, the Prosopididae eat pollen directly and regurgitate it for the brood. The leaf-cutter bees, Megachilidae, collect pollen ventrally. The Andrenidae can collect pollen in their hairy feet (legs).

Social bees are the most versatile, most active and best known of the pollinators. Bees are well adapted for the transportation of pollen. Much of the pollen is removed by grooming, but many flowers place pollen upon the bee in places where it cannot be removed easily. Up to 15,000 pollen grains have been counted from a single bee (Kendall & Solomon 1973).

Insect Migration and Foraging Resources

Many questions can be answered about the habits, distribution, migration and daily life of insects by knowing their migration and dispersal patterns, source zones and movement in and around local surroundings. Behavioral studies to determine the daily activities of insects is difficult, because the majority of insects are relatively small and following and watching them is difficult, time consuming and tedious. When insects migrate, tracking their routes and migratory patterns compound the difficulties of behavioral studies.

Identification of pollen found on or in an insect is used to determine the insect's feeding and migratory activities (Hendrix & Showers 1992; Gregg *et al.* 1993; Lingren *et al.* 1993, 1994; Berkhousen & Shapiro 1994; Loublier *et al.* 1994). Because some plants grow only in certain ecological zones or geographic locations, the identification of pollen from those plant species can be used to determine the geographical origin of the insect. The geographical origin is important because it indicates possible migration routes and foraging zones, especially when there is temporal and geographical variation in the distribution of the identified plant. For example, grapefruit, oranges, etc. (*Citrus*) are geographically restricted to the southern portions of the United States, Mexico, etc. Pollen from grapefruit and oranges was found on insects captured in Oklahoma. Since citrus is not grown in Oklahoma, these insects must have foraged on citrus flowers in an area where the plants occurred, then migrated over 835 km to Oklahoma (Lingren *et al.* 1993, 1994).

Pollen has been used to determine geographic origins since 1895 when it was demonstrated that the geographical origin of honey could be determined from identification of the pollen within the honey (Lieux 1969). Parker (1923) discovered that the source and geographic origin of the nectar being foraged could be identified from the pollen in the stomach contents of honeybees.

From the pollen found on several species of Lepidoptera, Mikkola (1971) determined that some of the insects examined migrated to Finland. Hendrix & Showers (1992) found that black cutworm [*Agrotis ipsilon* (Hufmagel)] and armyworm [*Pseudaletia unipuncta* (Haworth)] adults captured in Iowa and Missouri contained pollen from plants that only grew in South and Southwest Texas. The identification of this pollen indicated that these insects migrated over 1300 km. Likewise, Lingren *et al.* (1993, 1994) determined from pollen found on corn earworm, *Helicoverpa zea* (Boddie), adults captured in Oklahoma that they originated in South Texas.

Historically, pollen analyses of Lepidopteran species have been conducted by examining the insect's exterior body parts (eyes, proboscis, legs, etc.) (Wiklund *et al.* 1979, Courtney *et al.* 1982, Lazri & Barrows 1984, Lingren *et al.* 1993) (Fig. 1). Mikkola (1971) combed the Lepidopteran body over a glass slide and rolled the proboscis in euparal, then examined the slide with light microscopy (LM). Hendrix *et al.* (1987) and Turnock *et al.* (1978) also examined the exterior of adult Lepidoptera but used a combination of LM and scanning electron microscopy (SEM). Courtney *et al.* (1982) shortened the external examination process by using only SEM and examined the head, proboscis, palps and body. Bryant *et al.* (1991) found that SEM examination of just the proboscis (Fig. 2) and eyes was faster and more accurate than other techniques. Gregg *et al.* (1993) again shortened the external examination by splitting the proboscis and examining it with SEM. Loublier *et al.* (1994) dissolved the entire head, including antennae, in sulfuric acid and then examined the residue with LM.

Although Turnock *et al.* (1978) found pollen within the proboscis tube, they did not examine the crop (insect organ that is the receptacle for food) or other parts of the digestive system for pollen. The idea that pollen is drawn into the digestive system at the time of feeding has not been examined. Neither the Lepidopteran stomach nor crop contents have been previously examined for pollen; although, they have in other insects.

For years, scientists have needed to answer basic questions about insect pests, beneficial insects, their migration and migration routes, dispersal patterns and local wanderings around the cropping systems in order to develop control measures against these pests. More effective control methods can be developed when the insects' movements in and out of cropping areas, migration and dispersal patterns are known.

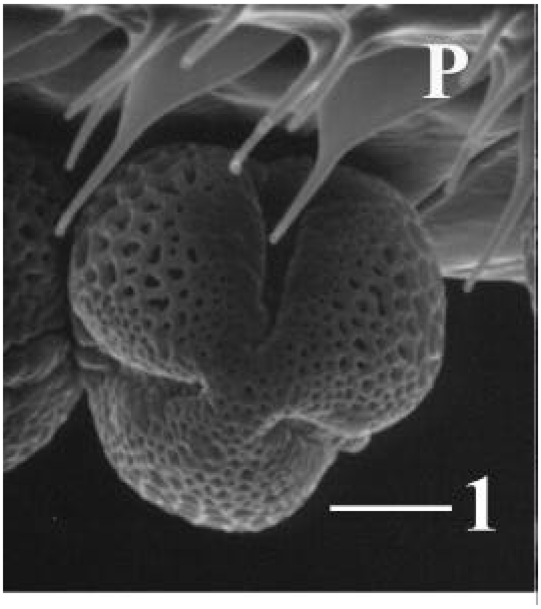

Figure 1. *S. nigra* pollen found on a beet armyworm adult, *Spodoptera exigua* (Hübner). The bar indicates 5 ìm and P = part of the proboscis. This pollen grain is in polar view. The pollen grain is tricolporate (contains three colpi and three pores) and reticulate (net-like).

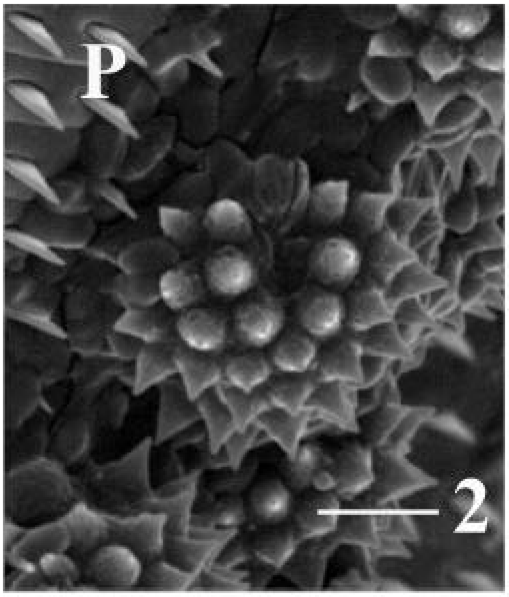

Figure 2. A pollen grain from the Asteraceae (sunflower) plant family found on a beet armyworm adult, *S. exigua*. The bar indicates 10 ìm and P = part of the proboscis. This pollen grain is also tricolporate (contains three colpi and three pores) and echinate (has processes).

Examining insects for a pollen marker from edaphically or geographically restricted taxa is often like looking for a "needle in a haystack." The majority of pollen found on or in insects is from taxa that are so widespread that the identification of that pollen cannot be used to determine geographical origin of the individual nor its migration. Because of the blooming patterns of many plants, insects may forage mainly on pollen from plants that are ubiquitous. In these cases, the pollen markers can be used to determine possible food sources, but not for extracting information about migration and dispersal.

For these reasons, many techniques have been developed to mark insects. Markers have included everything from the use of dyes, paints, etc. placed directly on the insect to spraying crops with foreign protein, dye, iridium, etc. (Bailey 1951, LeCren 1965, Begon 1979, Blower *et al.* 1981). Unfortunately, most of these marking techniques are extremely time consuming and tedious. The markers must be applied to the insect, the insect released and hopefully recaptured. In order for the marking technique to work, marked insects must be recaptured and re-examined to determine the type, color, etc. of the marker. Often dyes and paints kill the insect or interfere with its normal daily patterns rendering it useless.

The ideal marker is one that can be applied by the insect in question and does not easily come off. Markers should be easily recognizable, should not come off, should not be transferred from one individual to another and should not cause harm to the individual. Marking insects with paint or with a radioactive marker can be time consuming and labor intensive and can lead to injury or death to the subject being investigated. An ideal situation is to have the insects mark themselves in some way. This removes the human aspect and the trauma to the insect of marking. Many insects readily feed on sugar and water solutions.

Pollen is a natural marker for insects that forage on nectar, pollen or other plant secretions. Local and long distance migration and migration pathways can be determined from the identification of the pollen on these insects. If a pollen or spore type not normally found in the area is added to a sugar-water solution and put in or on something that attracts the insects being studied, then they would mark themselves as they fed on the sugar solution. Later the same day at different distances, these insects could be captured and examined for the marker that was placed in the sugar solution.

When insects feed on pollen and or nectar they become covered with pollen. From the identification of that pollen, the plants on which the insects fed can be determined. Data obtained can be used to determine the feeding sources of the insects. Once identified, the plant species can be tested for its attractiveness. If attractive to the insects, the plant's volatiles can be extracted and tested to determine if the volatiles will attract the insects. Once determined that the volatiles attract the insects, a bait can be formulated. If a killing agent is added, then the insects can be lured away from the location to another location where they are killed. This helps reduce the costs of spray application of insecticides and reduces the amount of insecticides put into the environment.

Honey

Honey is one of the oldest foods of mankind and there have been references to it and to the bees that gather it through out recorded history. Jacob sent his sons down into Egypt with a little balm and a little honey (Proctor & Yeo 1972). Today, honey is regarded as an important food and carbohydrate throughout the world. Honeybees utilize certain natural raw materials that are identifiable in honey. These raw materials include pollen and nectar. Pollen is the bee's major source of proteins. Nectar is a bee's source of carbohydrates.

One of the goals in the study of honey (melissopalynology) is to determine the sources used by honeybees to make honey. Identification of the pollen found in honey shows which plants honeybees visit to obtain the nectar to make honey.

Beekeepers need to know what flowers the bees visit so that they can locate the hives near the best flower sources. Optimal locations ensure the health and growth of the colony and honey for the beekeeper. In addition, honey of a dominant floral type (e.g., mesquite, clover, or citrus) can bring a higher price than honey of mixed or unknown floral sources.

Except for the USA, most major honey producing regions (Brazil, Canada, China, France, Great Britain, New Zealand, Spain, Switzerland, Japan and the former USSR) require three types of certification for honey and honey products that includes the verification of the honey's floral type, quality and precise place of origin. From the pollen in honey, the honey's floral type, quality and geographical origin can be assessed. The data collected from the pollen analyses of honey enable these nations to impose strict laws governing the importation and exportation of honey products. This certification requirement limits the exportation of United States domestic honey because the United States does not have this type of certification.

Climatic Changes

The change of climate patterns often can be determined by pollen analyses of the soil, especially the soil of bogs and lakes. Pollen falls onto the soil and into lakes and bogs throughout the year. Each year, another layer of pollen is added to the pre-existing layer. This process repeats year after year. As the vegetation changes, so does the pollen that is deposited. Thus, the vegetational changes are recorded in the different layers. By analyzing different layers, different pollens will be found. Any climate change can be determined by comparing the pollen found in the layers to the climate in which those plants occur. For example, if a core was taken from a pond in the desert. On analyzing the core, pollen from pines and firs were found. Although today that area is arid, the pollen record indicates that at one time the area supported the growth of pines and firs. Thus a climatic change had occurred from a cool, moist climate to one that was hot and dry.

Archaeological Palynology

Although von Post was the first to examine fossil pollen from archaeological soils (von Post *et al.* 1925), Iversen saw the full potential of pollen studies for archaeologists. By examining the fossil pollen collected in core samples from a bog, Iversen (1941) speculated how and when the local transformation from hunting and gathering to agriculture occurred. Iversen not only dated the introduction of agriculture in northern Europe, but also provided data concerning what plant species were introduced and how prehistoric groups altered the equilibrium of the natural vegetation by clearing the forest (Bryant & Holloway 1996).

Pollen evidence confirmed these types of data in other regions of Europe, the United States of America, Canada, Mexico and Japan (Godwin 1944, Mitchell 1951, Martin 1963, Durno 1965, Watts & Bradbury 1982, Bryant & Holloway 1983, Hall 1985, Tsukada *et al.* 1986, McAndrews 1988). Pollen analysis of soils recovered directly on top of a Neanderthal burial revealed unusually high pollen percentages and pollen clusters from alpine flowers (Lerio-Gourhan 1975). Because the pollen of these flowers is entomophilous, Lerio-Gourhan concluded that the flowers from nearby hillsides were placed in the Neanderthal's grave. Today, archeologists routinely collect soil samples from burials for pollen analysis. Data suggest that many prehistoric cultures had graveside rites (Bryant & Holloway 1996).

O'Rourke (1983) used pollen trapped in adobe bricks from an ancient southwestern pueblo site to show that various walls were constructed from different source materials and possible erected at different times. Pollen analyses are used for determining the probable function of baskets, ceramic vessels, bedrock mortars and milling stones. Pollen from storage foods such as maize, amaranth, cattail, etc., often adhere to the insides of baskets or become lodged in the weave (Bohrer 1968).

Pollen from coprolites (desiccated or mineralized feces) can provide information about the diet of prehistoric humans. Pollen can occur in coprolites through the eating of flowers or seeds or through the unintentional ingestion of pollen in medicinal teas or foods (Sobolik 1996). Pollen in this context is considered directly associated with food or a medicinal item. Thus, pollen analyses of coprolites offer direct clues to food items eaten intentionally. This type of precise information cannot be derived as accurately from other methods.

Palynological and palaeoethnobotanical data from underwater archaeological sites have been generally ignored. This lack of palynological data is possibly due to archaeologists not being trained to look for botanical remains associated with shipwrecks (Weinstein 1996). For example, underwater excavations, amphoras and similar ceramic containers are often emptied underwater to facilitate removal from the sea (Throckmorton 1960). Most investigations of shipwreck sites focus on technological innovations in ship design reconstruction of the vessel and retrieval of artifacts (Weinstein 1996). As a result, excavation and survey reports are often biased toward descriptions of macroscopic hull remains and cargo.

Forensic Palynology

Soil, leaf litter and even dust contain pollen grains that may provide clues to the type of vegetation, habitat or geographical location from which a sample originated. Soil from shoes, fingernails or just on the clothing may yield enough pollen to reconstruct the recent movements of a person or animal. Today, the country of New Zealand leads the world in the use of forensic palynology and the acceptance of this type of evidence in courts of law.

The following is an example of forensic palynology that is used with the authors' permission (Bryant & Mildenhall 1999) and comes from the website: http://www.crimeandclues.com/pollen.htm. During a vacation along the Danube River, a man disappeared near Vienna, but his body could not be found. The police had neither motive nor evidence to link the suspect with the possible crime. As the investigation proceeded, a search of the suspect's room revealed a pair of boots with mud still attached to the soles. The mud was examined and contained modern spruce, willow and alder pollen. In addition, there was a special type of 20 million-year-old fossil hickory pollen grain present in the mud.

Based on the pollen evidence, the area where the defendant must have walked when getting mud on his boots was pinpointed. Only one location, a small area 20 kilometers north of Vienna along the Danube Valley, had soils that contained the precise mixture of pollen in the mud. When confronted with the identity of this location, the shocked defendant confessed his crime and showed the authorities where he had killed the victim and then buried the body. The discovery of the murdered victim's body and the conviction of the criminal were based primarily on the evidence recovered from a pollen sample associated with the crime.

Medical Palynology and Aerobiology

Many people attribute a runny nose, watery itchy eyes, etc. as the common symptoms of allergies. About 15% of the people in the USA suffer from allergies caused to biological particles such as pollen (O'Rourke 1996). Pollen production varies among plants species. Each plant species disperse pollen at about the same time each year. High wind speed promotes the dispersal of anemophilous pollen. The distance anemophilous pollen travels away from the initial plant varies and often depends on the magnitude and direction of wind currents, height of the plant and density of the vegetation cover (Jarzen & Nichols 1996). Studies have shown that more pollen is found near and far away from the parent plant than in the middle (Willson 1983). Many wind-pollinated plants release their pollen only during favorable conditions such as low humidity. A single birch (*Betula*) catkin may produce as many as five million pollen grains (Proctor & Yeo 1973). Traverse (1988) estimated that a ten-year-old branch system of a pine (*Pinus*) produced 350 million pollen grains.

Medical palynologists are concerned with the interaction of pollen and spores with the human respiratory tract. Factors affecting deposition include particle size, particle density, the subject's activity level, etc. Pollen or spore allergens must be wind-borne, occur in large quantities, produce hay fever and be wide spread (Norman & King 1987). The almost ubiquitous aeroallergen, grass pollen, causes allergy symptoms worldwide. Closely related plants may have the same proteins (antigens) and cause the same allergic response. The medical community believes that aerobiologists have identified most of the common taxa that affect most people (O'Rourke 1996). Now it is up to the immunologists and biochemists to determine at what point a person shows symptoms to various pollen types.

Conclusion

Pollen is durable, distinctive and identifiable. It can be used in many applications and in many disciplines. The variety of uses of pollen analyses is infinite. Identification of the pollen found on or in an insect can help researchers determine long distant and local migration and dispersal, migration routes, food sources and source zones. This information is important for researchers studying pollination and field crops. Not only can pollen be used to determine these things for insect pests, but also for beneficial insects. By knowing about the habits and migration of both beneficial insects and insect pests, more effective control methods can be developed to control insect pests.

Techniques to isolate pollen from insect bodies or organs are relatively easy and inexpensive compared to those needed to extract pollen from soil and other substrates. Historically, light microscopy is used for the identification of pollen extracted from inside the insect and scanning electron microscopy for pollen found on the outside of the insect. Regrettably, scanning electron microscopy can be expensive, may not be available to researchers and may be time consuming if training is needed to operate the microscope. However, light microscopy is less expensive, available to most scientists, takes less training and can be used with almost equal results.

Research into the pollinators of crops, horticulture plants, orchards, etc. can benefit by determining what the pollinators are, what pollen they carry and what effect they have on plant yields. Likewise, pollen research on beneficial insects, not only can indicate what food sources beneficial insects use, but also can help determine their migration and habits. Addition of plants to help increase the number of beneficial insects near crops enables beneficial insects to multiply. Ultimate this reduces the amount of insecticide needed on the crop, reduces insecticide costs and increases the farmer's profit.

Most scientists are not trained in palynology. However, the benefits of pollen analyses out number the negative aspects. Hopefully, as the benefits of pollen analyses are realized, palynology will take its place as a major tool in research, especially in entomological research.

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