

Pollination Ecophysiology of *Mercurialis annua* L. (Euphorbiaceae), an Anemophilous Species Flowering all Year Round

MARCELLO LISCI*†, CATERINA TANDA* and ETTORE PACINI*‡

* Botany Section, Department of Environmental Biology, University of Siena, Via P. A. Mattioli 4, 53100 Siena, and † Department of Plant Biology, University of Florence, Via G. La Pira 4, 50121 Florence, Italy

Received: 20 December 1993 Accepted: 26 February 1994

Mercurialis annua L. is a dioecious anemophilous species that flowers all year round in central and southern Italy. The flowers of both sexes are dimorphic: the female flower has a vestigial calyx; the male flower consists only of a calyx that opens at anthesis. The anthers always dehisce after anthesis. The anthesis of male flowers seems to be temperature dependent, whereas anther dehiscence is related to relative humidity. The pollen grains vary in volume according to the season: they are smaller when relative humidity is low and *vice versa*. They always decrease in volume after anther dehiscence and have the capacity to vary in volume and reach equilibrium with a changing environment. Viability is high, but may drop suddenly during heavy rain or hail that damage the exposed male flowers. The number of pollen grains per stigma varies from 0 to 300. The data is discussed in relation to the type of pollination and environmental characteristics.

Key words: *Mercurialis annua*, dioecism, anthesis, anther dehiscence, pollen volume, pollen viability, anemophilous pollination, pollination ecology.

INTRODUCTION

The anthers may open before, after or at the same time as the flower (Pacini, 1992). If the anthers open before the flower, the pollen is only exposed once the flower opens; if the flower opens first, pollen exposure is limited by anther dehiscence (Pacini, 1992). Both anthesis and anther dehiscence are species-dependent and are influenced by environmental conditions (Richards, 1986). Hence the pollen of a given species is adapted to survive well in the plant's environment (Ottaviano and Mulcahy, 1989). The pollen dehydrates before being dispersed and rehydrates before germination so that the grains reach the female sporophyte in a viable state (Heslop-Harrison, 1979; Pacini, 1990a). Species with dehydrated and hence quiescent pollen adapt better to the environment during anther dehiscence, exposure to dispersing agents and transport (Pacini, 1990a).

Mercurialis annua L. (Euphorbiaceae) is a dioecious herbaceous plant of wide distribution (Meusel *et al.*, 1978). It is common throughout Italy, infesting gardens and vineyards, but also grows in quite arid areas (Pignatti, 1982). It is allergenic (Knox, 1979). Its flowering period varies according to its geographic location. In northern Europe and northern Italy, this period extends from mid March to mid September, whereas in Mediterranean countries such as central and southern Italy it flowers nearly all year round (Charpin and Surinyach, 1974).

The Euphorbiaceae are mostly pollinated by insects. In the genus *Euphorbia*, insects are attracted by extrafloral nectar in the so-called cyathium, a type of inflorescence

typical of the genus, in which the staminal and pistillate flowers are very close (Fægri and van der Pijl, 1979). *M. annua* is anemophilous (Hegi, 1965; Hesse, 1980), but there is some uncertainty about the existence of reduced nectaries in the female flower (Daumann, 1972).

M. annua has been studied for the determination of sex (Durand and Durand, 1985, 1991; Louis, Augur and Teller, 1990). Recently, seed dispersal and germination, which occur in different ways according to the season of the year, were studied (Pacini, 1990b). In fact the seeds germinate either when the elaiosome is removed by ants or when the germination inhibitor is removed by rain.

In this study we investigated the pollination ecology of this species under the different environmental conditions occurring throughout the year. A further paper will examine the question of development, morphology and receptivity of the stigma in relation to the seasons.

MATERIALS AND METHODS

Plant material

The plants were collected in 1987 in cultivated fields around Siena, where they are spontaneous. For the sake of homogeneity, all data on the reproductive characteristics of *M. annua* was obtained in male and female plants having a fresh weight between 51 and 75 g (Fig. 1, Table 1).

In order to determine the number of pollen grains per stigma and the number of pollen tubes per style we collected a stigma from every plant growing in a rectangle of land measuring 100 × 2 m. In this area, there were 54 female plants and 73 male plants.

‡ For correspondence.

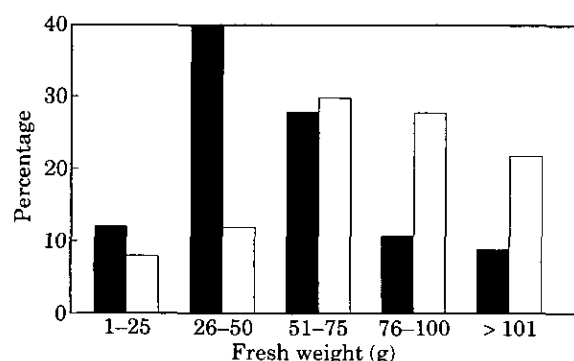


FIG. 1. Histogram of fresh weight of male (■) and female (□) plants of *Mercurialis annua*. Male plants have a lower mean weight than female plants.

TABLE 1. Principal reproductive characteristics of *Mercurialis annua*

	Mean $\pm$ s.d.	n	Range
Male inflorescences per plant	55 $\pm$ 11	115	137
Male flowers per inflorescence	58 $\pm$ 16	100	83
Male flowers per plant	3555 $\pm$ 123	100	1428
Length male inflorescences (mm)	55.2 $\pm$ 10.2	87	3
Length male flowers (mm)	1.3 $\pm$ 0.4	64	1.5
Stamens per male flower	10 $\pm$ 2	42	7
Pollen grains per anther	1312 $\pm$ 154	25	552
Pollen grains per male flower	13883 $\pm$ 988	25	9234
Female flowers per plant	104 $\pm$ 27	78	109
Length female flowers (mm)	3.4 $\pm$ 0.6	90	3

Some male plants were kept in pots out of the wind to study pollen volume and anthesis of the flowers and anthers.

Climate

Figure 2 is the temperature and rainfall diagram of Siena for 1987, based on data of the Italian Ministry for Public Works (Ministero Lavori Pubblici-Servizio Idrografico, 1968–1987) and presented according to Bagnouls and Gaussen (1953) and Walter and Lieth (1960).

Light microscopy

The morphological characteristics of the androecium and gynoecium were observed and measured with a stereomicroscope. The anthers were fixed in 3% glutaraldehyde in cacodylate buffer, dehydrated in ethylene glycol, ethanol and butanol, embedded in glycol methacrylate (Feder and O'Brien, 1968), and stained with: (a) PAS for total insoluble polysaccharides; (b) naphthol blue-black for total proteins; and (c) Lugol for starch (Jensen, 1962).

To count the number of pollen grains per anther, five anthers were allowed to dehisce in a Petri dish. Five millilitres of water were added to the shed pollen and the dish was placed in a moderate oven to evaporate the water so that the grains adhered to the dish ready for counting. The count was repeated five times.

The number of pollen grains per stigma and the number of pollen tubes were determined by taking stigmas the day after pollination, that is, when the stigma began to wilt and the ovary to grow. They were stained with Aniline Blue (Martin, 1959) and visualized by fluorescence microscopy on the basis of the autofluorescence of exine. The tubes were visualized by stain-induced fluorescence.

Scanning electron microscopy

Pollen samples were collected from mature anthers. Some were washed for 30 min in a 1:1 mixture of chloroform and

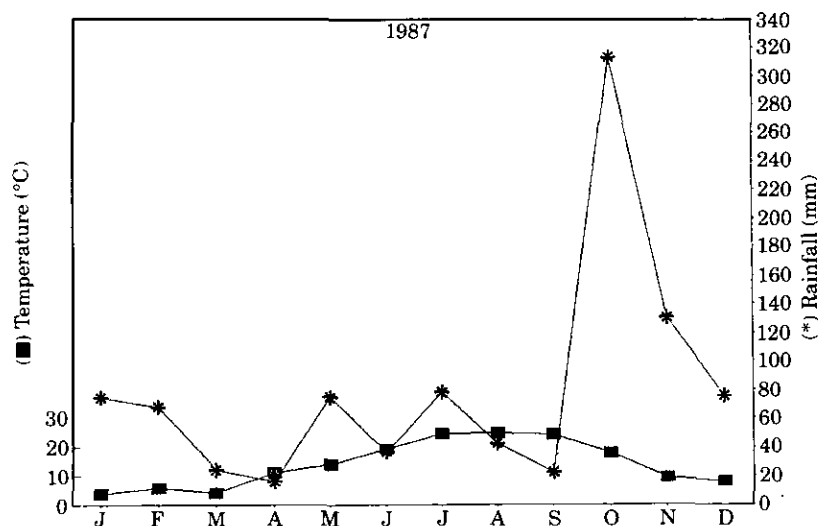


FIG. 2. Temperature and rainfall diagram of Siena in 1987.

TABLE 2. Calendar of observations of male flowers and anthers, and pollen measurement, in 1987

Observation no.	Date	Observation no.	Date
1	10 Jan. 1987	19	8 Jul. 1987
2	22 Jan. 1987	20	20 Jul. 1987
3	30 Jan. 1987	21	30 Jul. 1987
4	7 Feb. 1987	22	4 Aug. 1987
5	14 Feb. 1987	23	12 Aug. 1987
6	26 Feb. 1987	24	25 Aug. 1987
7	7 Mar. 1987	25	12 Sep. 1987
8	18 Mar. 1987	26	19 Sep. 1987
9	24 Mar. 1987	27	26 Sep. 1987
10	8 Apr. 1987	28	1 Oct. 1987
11	19 Apr. 1987	29	16 Oct. 1987
12	30 Apr. 1987	30	21 Oct. 1987
13	3 May 1987	31	4 Nov. 1987
14	18 May 1987	32	17 Nov. 1987
15	28 May 1987	33	28 Nov. 1987
16	4 Jun. 1987	34	2 Dec. 1987
17	15 Jun. 1987	35	15 Dec. 1987
18	27 Jun. 1987	36	28 Dec. 1987



FIG. 3. Male inflorescence of *Mercurialis annua*, showing flowers arranged in bunches. An open flower (arrow) shows three sepals. Bar = 1 cm.

carbon disulphide to remove the pollenkitt. The washed and unwashed samples were gold-coated in an Edwards evaporator and observed with a Philips 501 scanning electron microscope at 7.2 kV. The stigma was observed fresh.

Anthesis of male flowers and anther dehiscence

We labelled 65 male flowers in ten inflorescences on five different plants. Anthesis and anther dehiscence were determined every month for the whole year. Flower opening was observed with a magnifying glass. Anther opening was ascertained by touching the male flowers with a piece of rough black paper to detect pollen. At anthesis, temperature and relative humidity were monitored with a portable

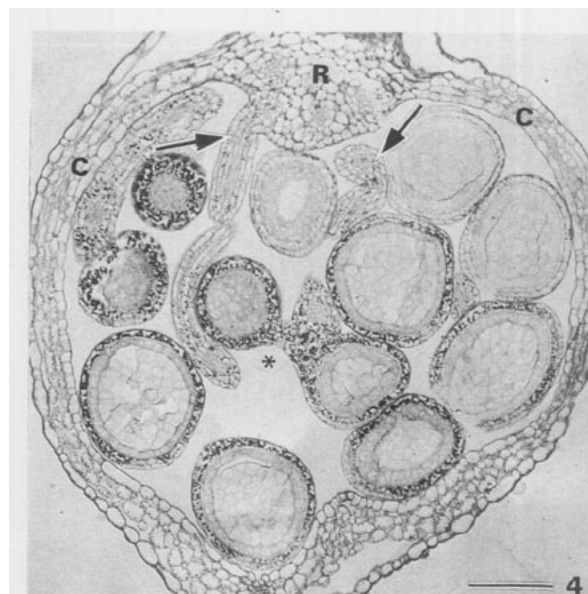


FIG. 4. Male flower bud with anthers at the pre-prophase stage. There are many anthers in the flower and each filament carries two lobes (*). The filaments (arrows) are inserted in the receptacle (R) along with the calyx (C). Many starch grains can be seen in the anther wall and in the filament (PAS). Bar = 250 μ m.

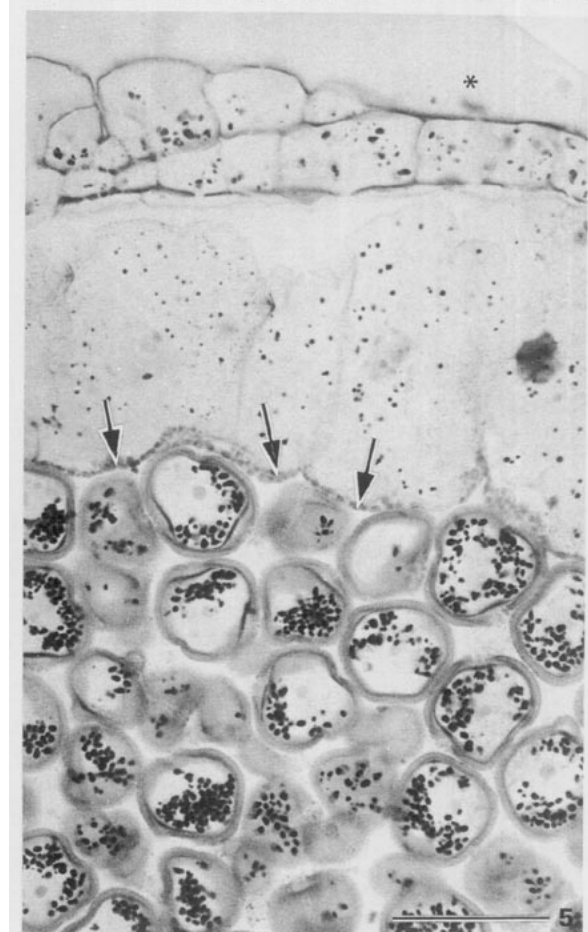


FIG. 5. Section of an anther at the late microspore stage. The locule is bounded by orbicules (arrows). The anther wall consists of two layers of cells, except at the stomium (*) where there is only one. Starch grains are visible in the microspores and the anther wall (PAS). Bar = 20 μ m.

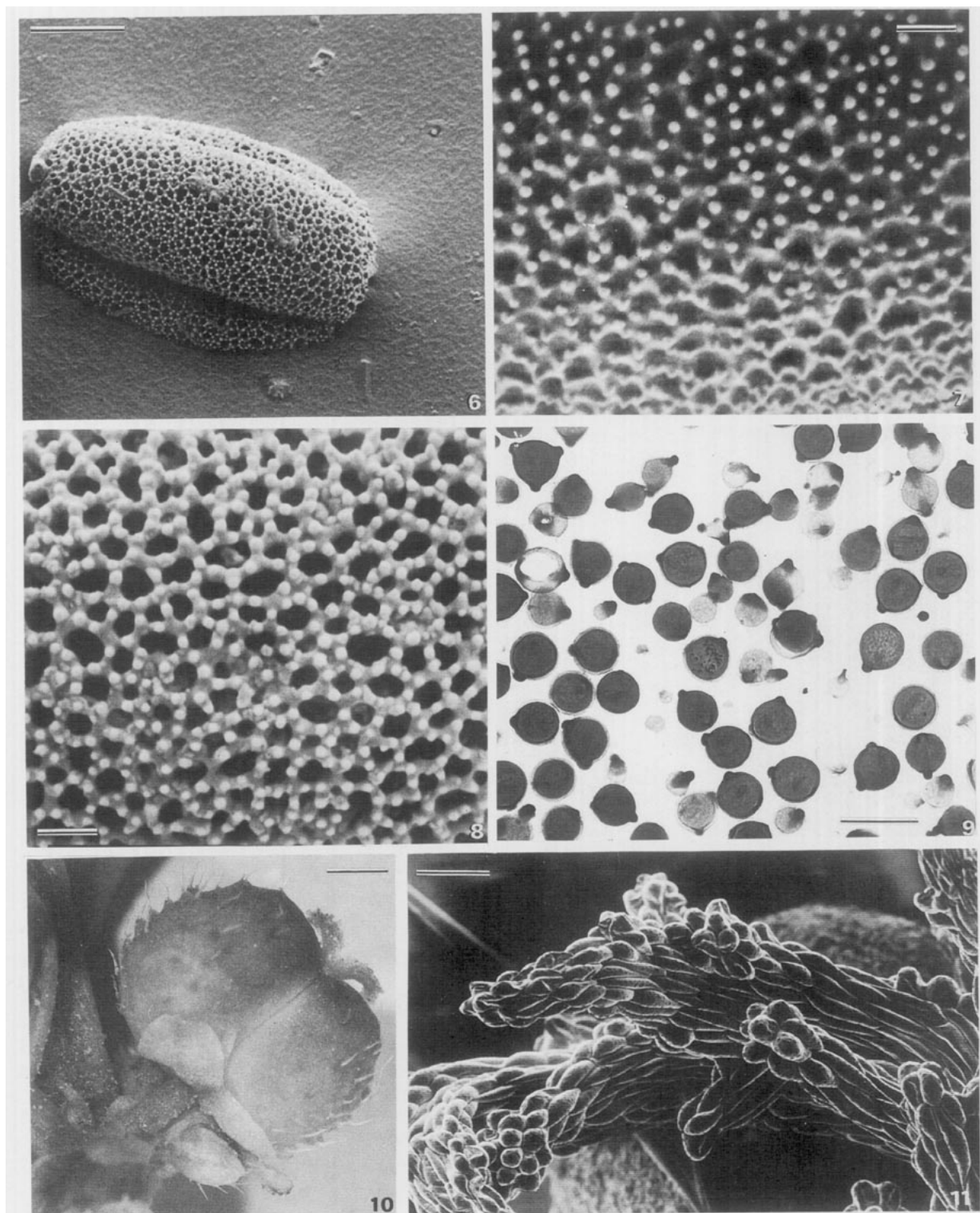


FIG. 6. Dry *Mercurialis annua* pollen is tricolporate with rough reticulate exine interspersed with spines. Bar = 5 μ m.

FIG. 7. Interpolar portion of the pollen surface. Pollenkitt fills the spaces of the reticulations. Bar = 0.5 μ m.

FIG. 8. Interpolar portion of the pollen surface washed with a 1:1 mixture of chloroform and carbon disulphide to remove pollenkitt. Bar = 0.5 μ m.

FIG. 9. Section of an anther just before dehiscence. The cytoplasm of the grain is PAS-positive. Bar = 50 μ m.

FIG. 10. Female flower: the ovary is bristly, the stigma dry, bilobate and feathery; the calyx is vestigial. Bar = 50 μ m.

FIG. 11. The stigma has closely juxtaposed protuberances. Bar = 50 μ m.

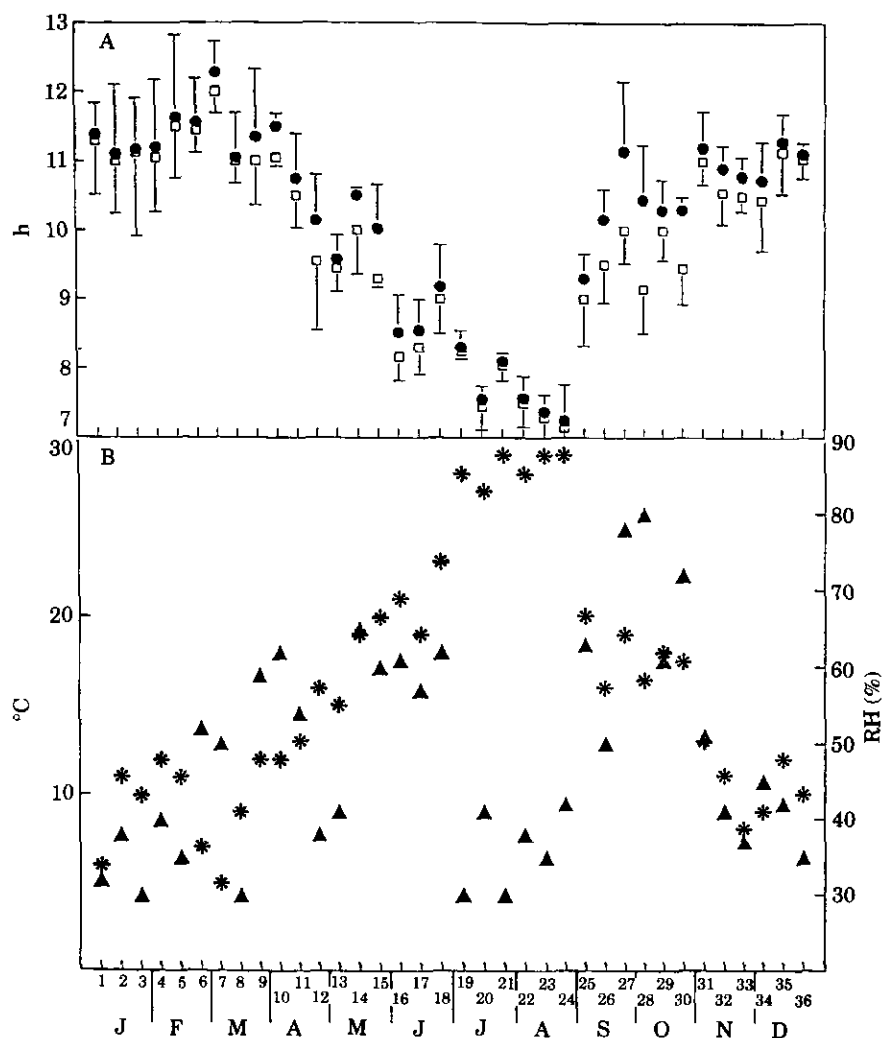


FIG. 12. A, Opening of male flowers (□) and anthers (●) in 1987. B, The temperature (*) and relative humidity (▲) are also reported. Bars represent standard deviation.

thermohygrometer. The observation dates are reported in Table 2.

Pollen volume

Pollen volume was calculated at anther dehiscence on the days listed in Table 2, and minutes and hours after dehiscence on 12 Jan., 14 Apr., 6 Jul. and 11 Oct. 1987. The pollen grains were collected by shaking the anthers onto a microscope slide. Immersion oil was added followed by a cover slip and the pollen was observed with a lanometer projecting microscope. Pollen volume was calculated by the formula $4/3 \pi a^2 b$, where a is half the equatorial axis and b is half the longitudinal axis. At each observation date, ten slides were prepared with pollen from ten plants. The slides were prepared on the site to avoid modification of the pollen water content as a consequence of environmental changes. The volume of 50 pollen grains was measured on each slide.

Pollen viability

Pollen viability at anther dehiscence was determined by the fluorochromatic reaction (Heslop-Harrison and Heslop-

Harrison, 1970; Heslop-Harrison, Heslop-Harrison and Shivanna, 1984). This type of observation was performed on days 1, 15 and 30 of every month of the year. Pollen samples were obtained from ten anthers of ten flowers of different plants. In each sample, 250 pollen grains were tested.

RESULTS

Mercurialis annua is a dioecious plant. The male to female ratio was 1:3 in the area and period of the present study. This is in disagreement with Lloyd (1974) who found a preponderance of female plants.

Pollen and androecium morphology

Quantitative data on the inflorescence and flowers are reported in Table 1. Male flowers are grouped in bunches (Fig. 3) and consist only of a calyx with filaments emerging from the receptacle. Each filament carries an anther with two lobes (Fig. 4) containing 1312 ± 154 pollen grains (Table 1). In the late microspore stage, the anther locule is bounded by orbicules (Fig. 5). The tapetum is of the parietal

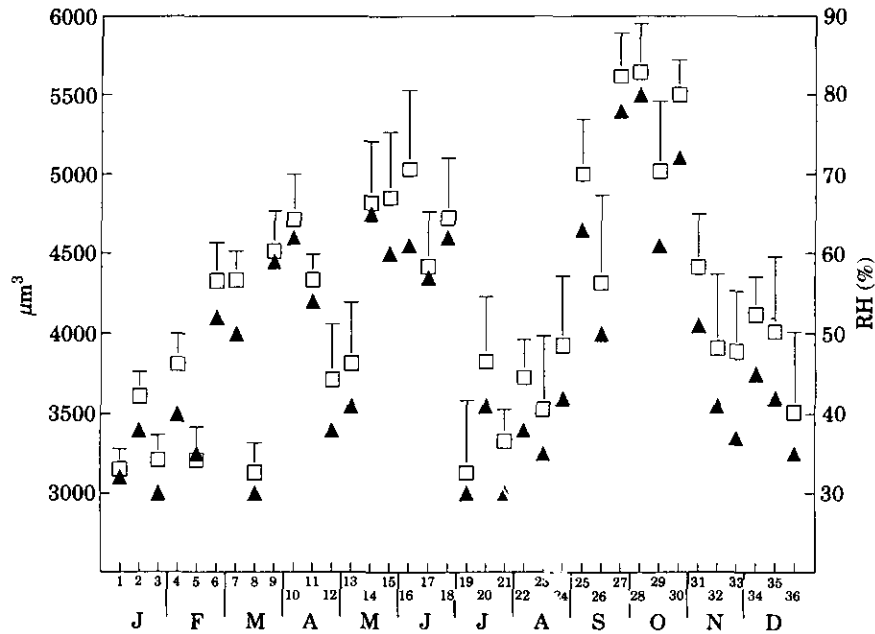


FIG. 13. Pollen volume (□) and relative humidity (■) through 1987. Bars represent standard deviation.

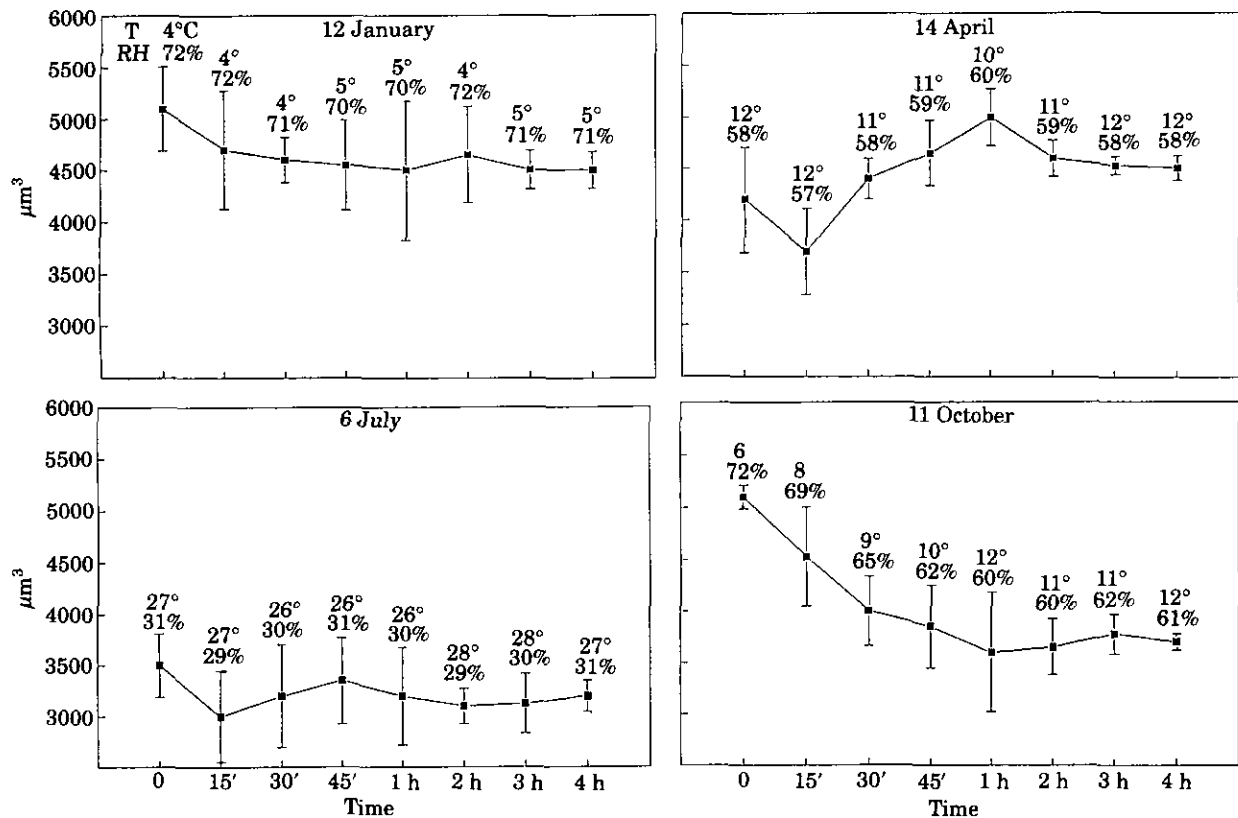


FIG. 14. Changes in pollen volume after anther dehiscence. The pollen grains always decreased in size immediately after anther dehiscence. Bars represent standard deviation.

type and has not yet begun to degenerate in the late microspore stage (Fig. 5).

The dry pollen grain is ellipsoidal. It is tricolporate with rough, reticulate exine interspersed with spines (Fig. 6). As

it degenerates, the tapetum forms the pollenkitt that covers the pollen before anther dehiscence, filling the reticulations (Figs 7, 8). The reserves of ripe pollen consist of cytoplasmic polysaccharides that are strongly PAS-positive (Fig. 9).

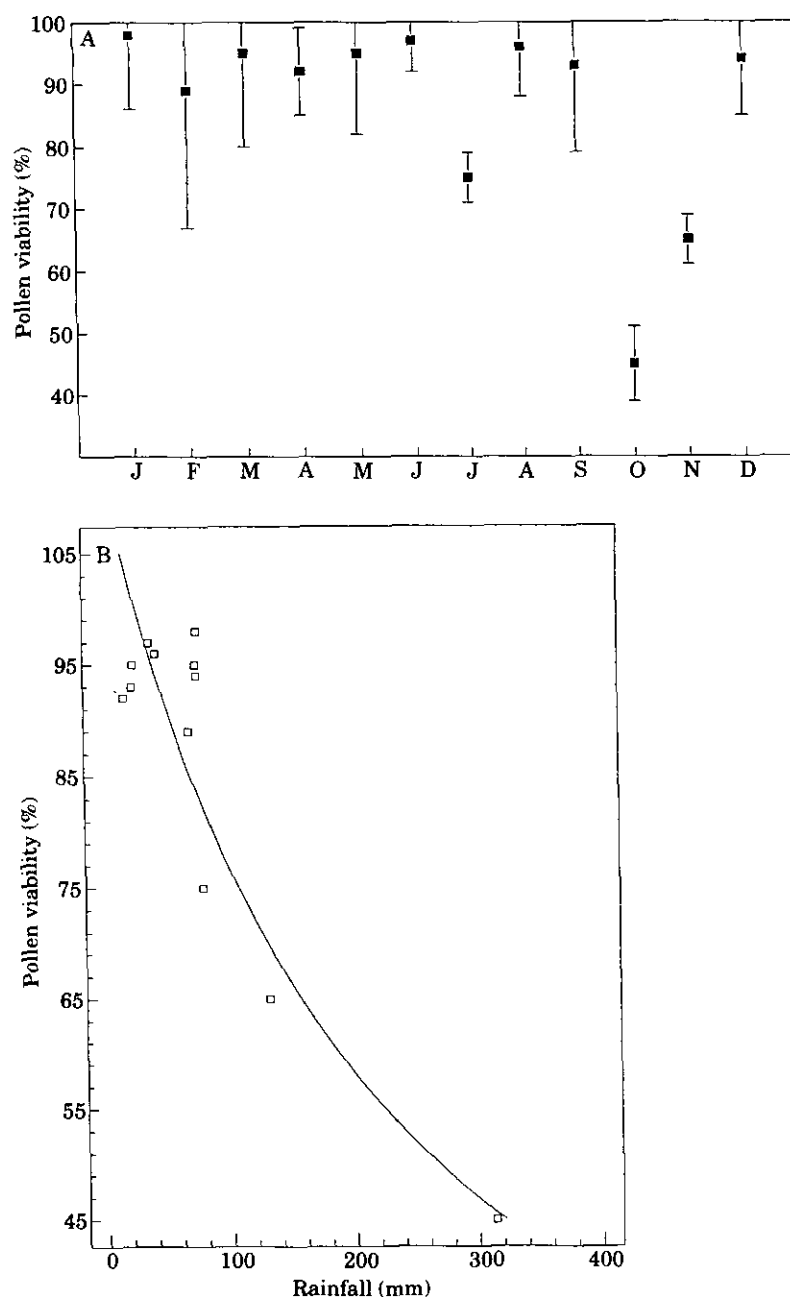


FIG. 15. A, Pollen viability in different seasons of 1987. Bars represent standard deviation. B, Regression analysis of pollen viability in relation to rainfall in 1987.

Gynoecium morphology

The female flowers are arranged at the top of the branch where the leaves are small; they are axillary, one to four in number and 3.4 ± 0.6 mm long (Table 1). They have a vestigial calyx that does not envelop the flower bud, and a bristly ovary containing two ovules (Fig. 10). Stigma differentiation is complete only when the female gametophyte is mature (preliminary data). The stigma is dry, bilobate, feathery and has closely juxtaposed protuberances (Fig. 11). Stigma receptivity lasts several days, depending on environmental conditions (preliminary data). The stigma begins to wilt shortly after pollination.

Anthesis of male flowers and anther dehiscence

The stamens, initially folded inside the bud, extend when the calyx opens. The wall of the anther consists of two cell layers and a single layer in the stomium, the point of least resistance where the anther opens (Fig. 5). In each inflorescence, three to four flowers open every day, usually distant from each other. The time of day of anthesis and anther dehiscence vary in time and seems to be influenced by temperature (the flowers) and relative humidity (the anthers) (Fig. 12).

In winter the flowers generally opened between 1100 h and 1200 h. The mean daily temperature recorded in this

period ranged from 5 to 12 °C, whereas relative humidity ranged from 30 to 59%. In spring the flowers opened between 0900 and 1000 h when the temperature was 12–23 °C and the relative humidity 38–65%. In summer they opened even earlier, between 0700 and 0800 h, when the temperature reached 27–29 °C with a relative humidity of 30–44%. In autumn, when temperatures were lower (16–20 °C) and relative humidity high (50–80%), they opened later (between 0900 and 1000 h) (Fig. 12).

The anthers always opened after the flower (Fig. 12). Under drier conditions, they opened 5–15 min after the flower; with high relative humidity they opened 15–90 min afterwards. The anthers wilted about 24 h after opening and the flowers fell from the plant 2 d after opening.

Exposure and dispersal of pollen

The pollen remained exposed until carried away by a breeze. This occurred within 6 h of anther dehiscence.

Pollen characteristics in the various seasons

Changes in pollen characteristics in relation to environmental conditions were observed in the different seasons.

Changes in volume at anther opening. Mean pollen volume was $3950 \pm 572 \mu\text{m}^3$, varying greatly from season to season (Fig. 13). The lowest values ($3000\text{--}4000 \mu\text{m}^3$) were recorded in the driest periods (RH 30–50%), namely Jan., Jul. and Aug. The highest values ($5000\text{--}6000 \mu\text{m}^3$) were recorded when RH was high (70–85%), mostly in Sep. and Oct. (Fig. 13).

These changes in volume were also observed in the minutes and hours after anther dehiscence (Fig. 14). On 12 Jan., pollen volume at anther dehiscence was $5100 \mu\text{m}^3$ at 4 °C and 72% RH. After 15 min the volume was $4650 \mu\text{m}^3$, and after 1 h, $4500 \mu\text{m}^3$, subsequently recovering to $4600 \mu\text{m}^3$. From this moment volume varied slightly (Fig. 14). On 14 Apr., pollen volume was $4200 \mu\text{m}^3$ at 12 °C and 58% RH when the anthers opened. It dropped to $3700 \mu\text{m}^3$ after 15 min, rising to $4400 \mu\text{m}^3$ at 30 min and $5000 \mu\text{m}^3$ after 1 h. In the hours that followed it dropped again (Fig. 14). On 16 Jul. (27 °C; 31% RH), the initial volume was $3500 \mu\text{m}^3$, dropping to $3000 \mu\text{m}^3$ and subsequently varying slightly around these values (Fig. 14). On 11 Oct. (6 °C, 72% RH), the initial volume was $5200 \mu\text{m}^3$ dropping to $3600 \mu\text{m}^3$ after 1 h. In the second and subsequent hours volume was substantially constant (Fig. 14).

Pollen viability. The pattern of viability at anther dehiscence in the different seasons (Fig. 15A) shows that *M. annua* pollen viability was always high, normally exceeding 90%. The graph shows only two drops to 65 and 75% in Nov. and Jul. respectively. These drops occurred after heavy rain or hail. Regression analysis between pollen viability and rainfall showed a correlation ($P < 0.001$; $r = 0.95$) (Fig. 15B).

Number of pollen grains per stigma and tubes per style. The number of pollen grains landing on the stigma varied widely from 0 to 300 grains per stigma (Fig. 16) with a peak

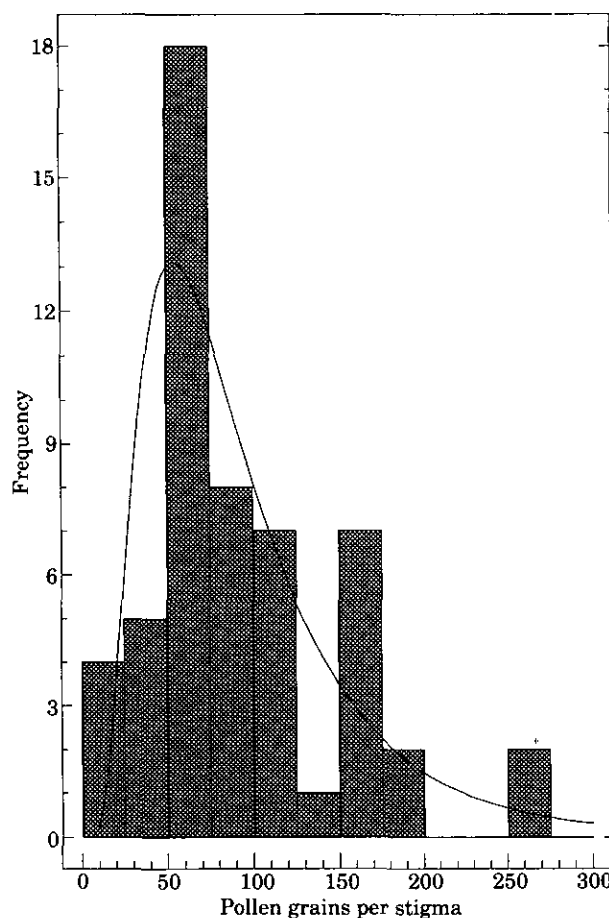


FIG. 16. Log-normal distribution of number of pollen grains per stigma.

between 50 and 75. The distribution was approximately log-normal ($\chi^2 = 4.04$; d.f. = 3; $P = 0.25$). The number of grains germinating on the stigma varied from 1 to 75: the more landed on the stigma, the more germinated (Fig. 17).

DISCUSSION

Anthesis of male flowers and anther dehiscence

Flower and anther dehiscence are influenced by environmental conditions. Optimum conditions for anthesis vary from species to species (Richards, 1986). In *M. annua* we observed that anthesis of male flowers was influenced by temperature throughout the year. At low temperatures (5–13 °C), flower anthesis occurred later in the day whereas at higher temperatures it occurred earlier.

The anthers, on the other hand, may open before, after or at the same time as the flower (Pacini, 1992). In *M. annua* the anther always opened after the flower. This process was influenced by the relative humidity of the flower's environment. The interval between the two events varied: in dry conditions (RH 30–40%), when anther dehydration was facilitated, the anthers opened within 5–15 min of flower anthesis; at moderate RH (41–60%) the anthers opened after 15–30 min and at high RH (61–80%), anther dehydration was further retarded and anther dehiscence

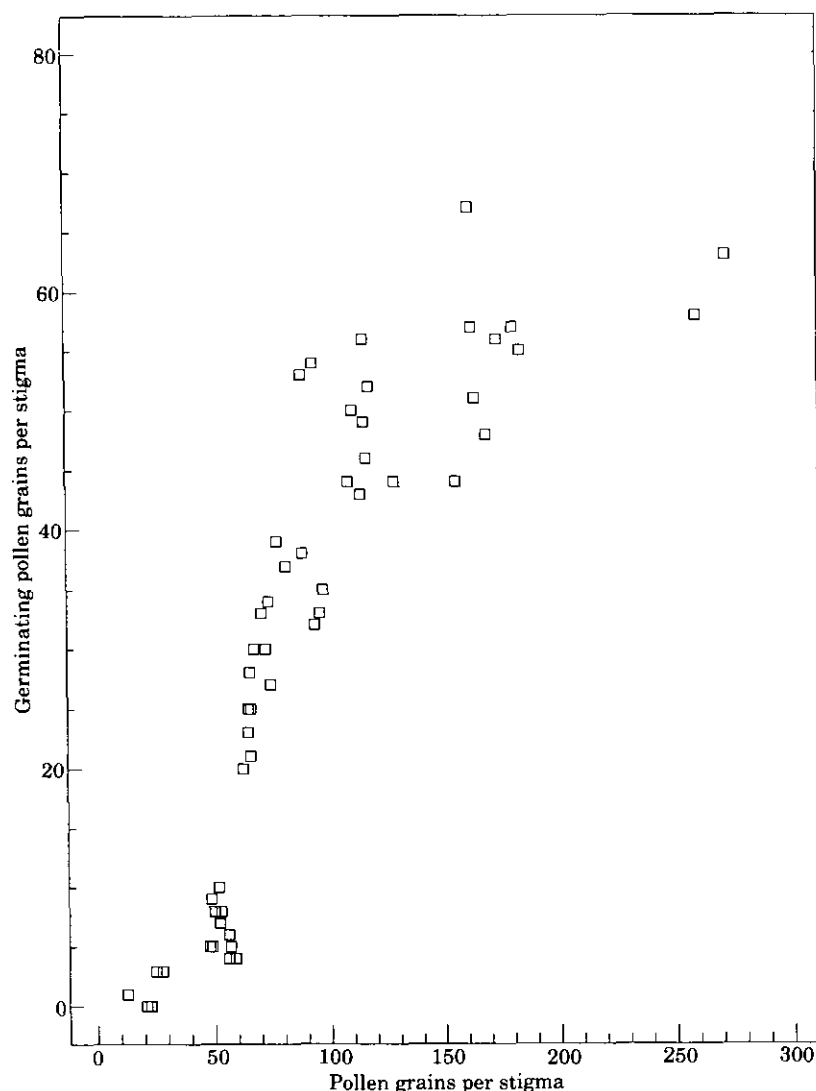


FIG. 17. Scatter plot of the number of pollen grains per stigma and the number that germinated.

occurred up to more than an hour after opening of the flowers.

Anthers open due to dehydration of the anther wall that leads to opening of the lobes (Stanley and Linskens, 1974). In general the mechanisms of dehydration involve evaporation of most of the water contained in the anther or migration of water to other parts of the flower (Heslop-Harrison, Heslop-Harrison and Reger, 1987; Pacini, 1990a). In *M. annua* dehydration can take place by both mechanisms. Reabsorption occurs when the weather is damp and this is why the interval between anthesis and anther dehiscence may be as long as 30 min. Evaporation occurs when the atmosphere is dry and the interval may be as short as a few minutes.

Pollen characteristics in the different seasons

Ripe *M. annua* pollen has cytoplasmic reserves of polysaccharides, probably of low molecular weight (Pacini and Franchi, 1988), and sugars (Hoekstra and van Roekel, 1988). Starch was not found at any time of the year. In other

long-flowering species like *Parietaria judaica* L., the percentage of starch in ripe pollen differs from season to season (Franchi, Pacini and Rottoli, 1984).

The volume of the pollen grains of *M. annua* is closely related to relative humidity, decreasing as soon as the pollen grains are exposed to air. After this initial decrease the pollen adapts to environmental conditions outside the anther. This is important because it shows that the pollen of this species that flowers all year round achieves equilibrium with its environment. The initial decrease in volume is probably due to partial dehydration.

Dehydration of the pollen grain is necessary for quiescence so that it can reach the female gametophyte fully viable (Wodehouse, 1935; Pacini, 1990a). Pollen quiescence is not stable in the sense that at anther dehiscence the pollen is not in equilibrium with the external environment, but quickly becomes so. This is very evident in *M. annua* pollen that adjusts to any relative humidity, maintaining high viability (Bassani, Franchi and Pacini, 1994). This allows it to remain viable for hours, whereas pollen such as that of *Cucurbita pepo* loses its viability very quickly (Nepi and Pacini, 1993).

Bassani *et al.* (1994) found a correlation between changes in volume and viability in different anemophilous and entomophilous species, including *M. annua*. They noted that 40% of *M. annua* pollen grains are still viable after 6 h at 98% relative humidity. This is not surprising as flower anthesis and anther dehiscence occur when relative humidity is still high. In fact, the flowers open from 1100 to 1200 h in winter and at 0700–0800 h in summer, i.e. at a time of day when humidity is still high. If the weather is dry, the anthers open immediately after opening of the flower; if the weather is damp, they open later.

In general *M. annua* pollen viability is high. Lower viability, which occurs after heavy rain and hail, is probably due to plant damage. In fact in relation to rainfall, viability dropped to about 45% in Oct. when there was 300 mm of rain. Also in Jul., usually a dry month, there was a slight drop in viability due to unseasonal rain. Viability probably decreases with heavy rain because *M. annua* is a herbaceous plant and quite fragile. The male inflorescence is terminal and hence unprotected. When the plant is damaged it uses its energy to repair its vegetative part in preference to its flowers.

Pollination

Species of the genus *Euphorbia* are pollinated by insects that are attracted by extrafloral nectaries in the cyathium (Fægri and van der Pijl, 1979). In the case of *M. annua*, pollination is anemophilous (Hesse, 1980) and our observations revealed the typical general characteristics of anemophilous species rather than those of species pollinated by insects. The male inflorescence is erect and terminal, designed to maximize exposure of the pollen to the wind and to avoid interception by the leaves of the mother plant. In larger plants, the pollen has a greater possibility of being broadcast. This means that older and larger plants can disperse pollen over greater distances.

The female flower is designed to maximize pollen collection. The flowers are arranged at the top of branches, with the stigma exposed to the wind. The stigma is feathery and dry like in other anemophilous species (e.g. the Poaceae) and unlike entomophilous species of the same family (Heslop-Harrison, 1992). This means that when there are anemophilous and entomophilous species in a family, the stigma differs in relation to the type of pollination.

The pollen has little sculpturing and there are orbicules, an indicator of anemophily (Pacini and Franchi, 1993). At anther dehiscence, the pollen is not released at once, as in the Poaceae, or launched into the air as in *Parietaria judaica* (Franchi *et al.*, 1984), but is dispersed within 6 h. A few pollen grains may be released when the calyx opens and the stamens unfold. In entomophilous plants, on the other hand, pollenkit keeps the pollen on the anther until the insect arrives, as in *Cucurbita pepo* (Nepi and Pacini, 1993). All this means that when anemophilous and entomophilous species coexist in a family, the function of pollenkit depends on the type of pollination (Hesse, 1980; Pacini and Franchi, 1993).

In *M. annua*, the distribution of the grains on the stigma is log-normal, as is typical in wind-pollinated species with

few ovules. In insect-pollinated species with many ovules (Uma Shaanker and Ganeshaiah, 1990) and in buzz-pollination (Buchmann, 1983), several models of distribution are possible. Gametophyte competition depends on the number of pollen grains landing on the stigma and on the number of ovules (Heslop-Harrison, Heslop-Harrison and Reger, 1985). In fact in *M. annua* the number of grains germinating increases in relation to the number of grains landing on the stigma.

ACKNOWLEDGEMENTS

We thank Prof. G. G. Franchi and Dr E. Pierson for their critical reviews of the manuscript of this paper; F. Ciampolini for technical assistance. Research performed under CNR program 'Reproductive Ecology'.

LITERATURE CITED

- Bagnouls F, Gaussen H. 1953. Saison sèche et indice xéothermique. *Bulletin de la Société d'Histoire Naturelle de Toulouse* 88: 193–239.
- Bassani M, Franchi GG, Pacini E. 1994. Humidity stress responses in pollen of anemophilous and entomophilous species. *Grana* 33 (in press).
- Buchmann SL. 1983. Buzz pollination in angiosperms. In: Jones CE, Little RK, eds. *Handbook of experimental pollination biology*. New York: Van Nostrand Reinhold, 73–113.
- Charpin J, Surinych R, eds. 1974. *Atlas Européen des pollens allergisants*. Paris: Sandoz Editions.
- Daumann E. 1972. Zur Blütenmorphologie und Bestäubungsökologie von *Mercurialis L.* *Preslia* 44: 308–315.
- Durand B, Durand R. 1991. Sex determination and reproductive organ differentiation in *Mercurialis*. *Plant Science* 80: 49–65.
- Durand R, Durand B. 1985. *Mercurialis*. In: Halevy HA, ed. *Handbook of Flowering*. Florida: CRC Press Boca Raton, 3: 376–387.
- Fægri K, van der Pijl L. 1979. *The principles of pollination ecology*. Oxford: Pergamon Press.
- Feder N, O'Brien PP. 1968. Plant microtechnique: some principles and new methods. *American Journal of Botany* 55: 123–142.
- Franchi GG, Pacini E, Rottoli P. 1984. Pollen grain viability in *Parietaria judaica* L. during the long blooming period and correlation with meteorological conditions and allergic diseases. *Giornale Botanico Italiano* 118: 163–178.
- Hegi G. 1965. *Illustrierte Flora von Mitteleuropa*. München: Carl Hanser Verlag, Band V, Teil 1, 126–129.
- Heslop-Harrison J. 1979. An interpretation of the hydrodynamics of pollen. *American Journal Botany* 66: 737–743.
- Heslop-Harrison J, Heslop-Harrison Y. 1970. Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescein diacetate. *Stain Technology* 45: 115–120.
- Heslop-Harrison J, Heslop-Harrison Y, Shivanna KR. 1984. The evaluation of pollen quality and a further appraisal of the fluorochromatic (FCR) test procedure. *Theoretical and Applied Genetics* 67: 367–375.
- Heslop-Harrison JS. 1992. The angiosperm stigma. In: Cresti M, Tiezzi A, eds. *Sexual plant reproduction*. Berlin: Springer-Verlag, 59–68.
- Heslop-Harrison JS, Heslop-Harrison Y, Reger BJ. 1987. Anther-filament extension in *Lilium*: potassium ion movement and some anatomical features. *Annals of Botany* 59: 505–515.
- Heslop-Harrison Y, Heslop-Harrison J, Reger BJ. 1985. The pollen-stigma interaction in the grasses. 7. Pollen-tube guidance and the regulation of the tube number in *Zea mays* L. *Acta Botanica Neerlandica* 34(2): 193–211.
- Hesse M. 1980. Ultrastruktur und Entwicklungsgeschichte des Pollenkitts von *Euphorbia cyparissias*, *E. palustris* und *Mercurialis perennis* (Euphorbiaceae). *Plant Systematics and Evolution* 135: 253–263.
- Hoekstra FA, van Roekel T. 1988. Desiccation tolerance of *Papaver*

- dubium* L. pollen during its development in the anther. *Plant Physiology* 88: 626–632.
- Jensen WA. 1962. *Botanical histochemistry*. San Francisco: W. H. Freeman and Co.
- Knox RB. 1979. *Pollen and allergy*. Studies in Biology, No. 107. London: Edward Arnold.
- Lloyd DG. 1974. Female-predominant sex ratios in angiosperms. *Heredity* 32: 34–44.
- Louis JP, Augur C, Teller G. 1990. Cytokinins and differentiation processes in *Mercurialis annua*. *Plant Physiology* 94: 1535–1541.
- Martin FW. 1959. Staining and observing pollen tubes in the style by means of fluorescence. *Stain Technology* 34: 125–128.
- Meusel H, Jäger E, Rauschert S, Weinert E. 1978. *Vergleichende Chorologie der zentraleuropäischen Flora*. Jena: Fischer Verlag.
- Ministero Lavori Pubblici-Servizio Idrografico. 1968–1987. *Annali Idrologici*. Roma: Istituto Poligrafico dello Stato.
- Nepi M, Pacini E. 1993. Pollination, pollen viability and pistil receptivity in *Cucurbita pepo*. *Annals of Botany* 72: 527–536.
- Ottaviano E, Mulcahy DL. 1989. Genetics of angiosperm pollen. *Advances in Genetics* 26: 1–64.
- Pacini E. 1990a. Harmomegathic characters of *Pteridophyta* spores and *Spermatophyta* pollen. *Plant Systematics and Evolution* (Supplement 5): 53–69.
- Pacini E. 1990b. *Mercurialis annua* L. (Euphorbiaceae) seed interactions with the ant *Messor structor* (Latr.), hymenoptera: Formicidae. *Acta Botanica Neerlandica* 39(3): 253–262.
- Pacini E. 1992. Transport mechanism of pollen—A short review. In: Cresti M, Tiezzi A, eds. *Sexual plant reproduction*. Berlin: Springer-Verlag, 69–79.
- Pacini E, Franchi GG. 1988. Amylogenesis and amyolysis during pollen grain development. In: Cresti M, Gori P, Pacini E, eds. *Sexual reproduction in higher plants*. Berlin: Springer-Verlag, 181–186.
- Pacini E, Franchi GG. 1993. Role of the tapetum in pollen and spore dispersal. *Plant Systematics and Evolution* (Supplement 7): 1–11.
- Pignatti S. 1982. *Flora d'Italia*. Bologna: Edagricole, 2: 28.
- Richards AJ. 1986. *Plant breeding systems*. London: George Allen & Unwin.
- Stanley RG, Linskens HF. 1974. *Pollen: biology, biochemistry and management*. Berlin: Springer Verlag.
- Uma Shaanker R, Ganeshaiah KN. 1990. Pollen grain deposition patterns and stigma strategies in regulating seed number per pod in multi-ovulated species. In: Bawa KS, Hadley M, eds. *Reproductive ecology of tropical forest plants*. Paris: Unesco, 7: 165–178.
- Walter H, Lieth H. 1960. *Klimadiagramm Weltatlas*. Jena: Fischer Verlag.
- Wodehouse RP. 1935. *Pollen grains*. New York: McGraw-Hill.