Comparative Medicine

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Comparing allergenic pollen exposure on horse grounds and the urban human environment.

A collaboration study with the Austrian Pollen Information Service.

MASTER THESIS

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1. INTRODUCTION

1.1. Background

This thesis is based on a previous study on allergy testing in horses using the ISAC131 microarray test which provided us with knowledge about the Immunoglobulin E (IgE) profiles in horses. IgE is an immunoglobulin that, if directed against specific allergens, can cause allergic symptoms. Allergens are denominated by an international code, e.g. birch (Betula verrucosa) major allergen number 1 is termed Bet v 1. The results revealed that the tested horses reacted to fungal allergen Alt a 1, worm Ani s 1, cockroach Bla g 1, earth wasp venom Pol d 5 and M. sympodialis Mal s 10 and Mal s 12 allergens. They also reacted via IgE to bee Api m 1, canine Can f 3, and in an auto-reactive manner to horse Equ c 1. In the category of food allergens, horses reacted via IgE to apple allergen Mal d 1, to Pen m 2, Pen m 4 and Pen m 11 from shrimp. Among pollen allergens, Aln g 1 of alder, Cyn d 1 of Bermuda grass, Phl p 4 of timothy grass and weed pollen allergen Che a 1 are important for horses. 70 percent of the tested horses had IgE to Fag e 2 from buckwheat, with the highest prevalence in recurrent airway obstruction (RAO) patients [1]. This finding proposed that buckwheat could be either an aeroallergen or a food allergen in horses, like in humans. Overall, the study revealed that the sensitization profiles in humans and horses are different, likely due to the different exposure. This prompted the present investigation.

1.2. Allergic reaction mechanisms

Allergies have been classified in four categories, according to the pathological mechanisms by Gell and Coombs 1963.

1.2.1. Type I Hypersensitivity: IgE mediated allergic reaction

This type of allergic reaction is a very fast one, in a sensitized individual, it occurs several minutes after encounter of the allergen.

However before, specific sensitization and formation of IgE antibodies must take place: It is commonly accepted that allergens elicit danger signals to the immune system that cause a bias

towards IgE production. Typical danger signals are barrier disruptions such as by enzymatic allergens, or by toll like receptor (TLR) 4 binding capacity of allergens. Then potential antigen-presenting cells (APC) of the mucosa or skin surfaces take up the allergen. The APCs including dendritic cells and B cells, recognise the antigen by innate immune receptors, but also surface expressed or bound immunoglobulins can be involved in this recognition. In the lymph nodes APCs present antigen peptides via major histocompatibility complex (MHC) II to naive T cells. The antigen-specific T cells get activated and evolve to T-helper cells 2 (Th2), if in addition a danger signal typically associated with allergens is present. These Th2 cells release several interleukins (IL), such as IL-4 or IL-13, which activate the isotype switch and IgE synthesis and secretion in the B cells. The secreted IgE binds then to effector cells, like mast cells, basophils, eosinophils, dendritic cells and monocytes. All these processes take place in the silent sensitization phase, still without any symptoms.

If a second contact occurs, the allergen cross-links cell-bound IgE which leads to the immediate release of mediator substances, for example histamine, which evoke the allergic reaction and symptoms. This is called the effector phase.

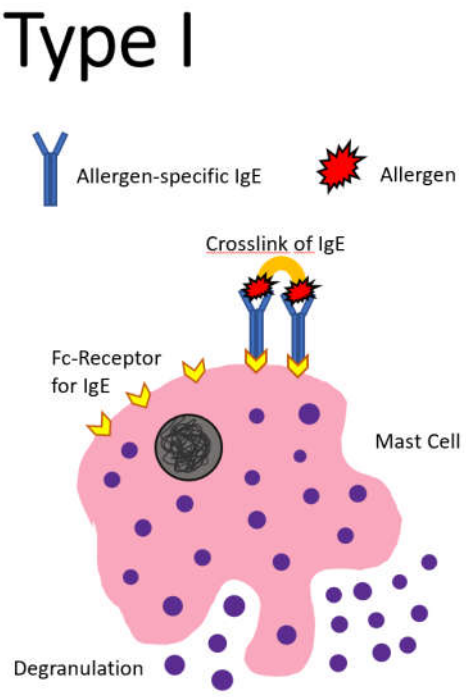

Figure 01: Scheme of type I hypersensitivity reactions, adapted from [2].

Subsequently, a late reaction to allergens can occur 6 – 8 hours in delay, caused by leukotrienes and infiltrating inflammatory cells. IgE in humans, in mice IgE and IgG1, are the key molecules in type I allergic reactions. These antibodies occur in mammals only. The plasma IgE levels differ depending on the species, but in general they are low, as most IgE is bound on the effector cells [3]. IgE can bind the original genuine allergen, but also structurally similar allergens. This phenomenon is called IgE cross-reactivity.

1.2.2. Type II Hypersensitivity: IgG or IgM mediated allergic reaction

The second type of allergic reactions is based on IgG or IgM, which activate the complement system, a highly conserved system of serum proteins causing inflammation. The antigen – antibody complex is fixed on cells expressing the specific antigen which then via complement activation leads to cytotoxic reactions. Therefore, the associated symptoms depend on which cells are targeted by the antibodies and destroyed [3].

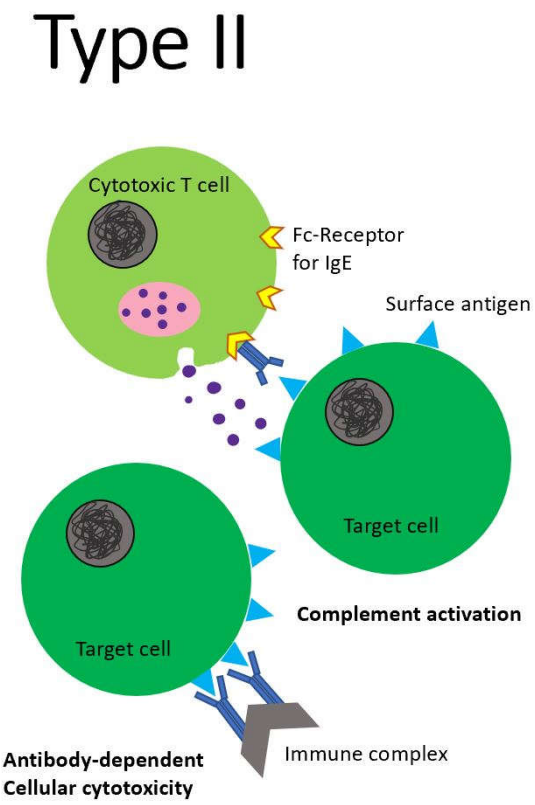

Figure 02: Scheme of type II hypersensitivity reactions, adapted from [2].

1.2.3. Type III Hypersensitivity: IgG, IgA and IgM mediated allergic reaction

The type III allergic reaction is based on blood-born immune complexes formed by IgG, IgA and IgM antibodies and soluble antigen. In the spleen innate immune cells usually clear out such immunocomplexes (IC). However, in settings of IC overload due to excess antigen, of complement defects or impairments of splenic clearance, they accumulate and activate the complement system, which results in systemic inflammatory response and fever. Clinical symptoms arise after 7 – 12 days and include endocarditis, arthritis or vasculitis, glomerulonephritis, or enteritis. Type III allergic reactions mostly occur due to aeroallergens especially in large domestic animals or to food allergens, like gliadin from gluten [3].

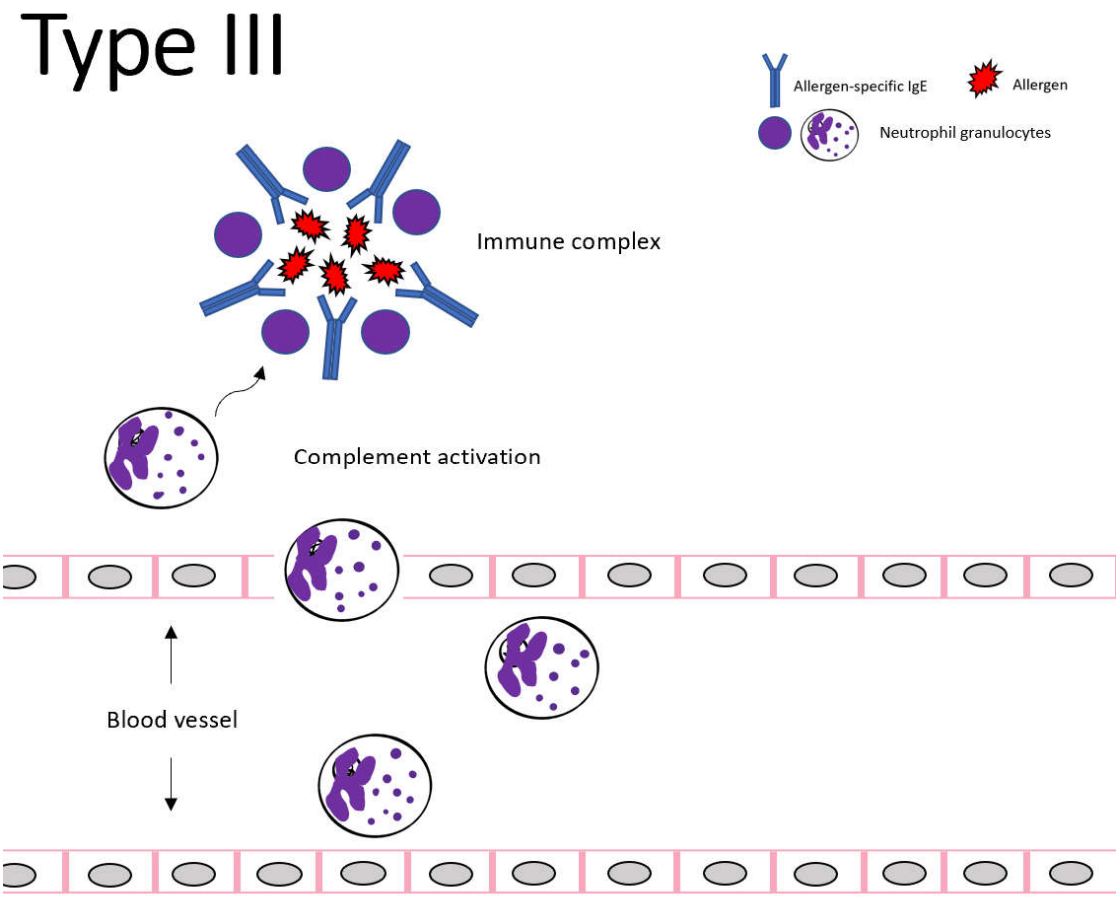

Figure 03: Scheme of type III hypersensitivity reactions, adapted from [2].

1.2.4. Type IV Hypersensitivity: allergic reaction mediated by specific T-cells

This type of allergic reaction is also called the "delayed -type hypersensitivity" (DTH), because symptoms occur after 24 – 72 hours after antigen encounter. Antigens are typically small molecules, like metal ions or chemicals. This reaction is based on specific T cells, which can release cytokines or evoke cytolytic or direct toxic effects on cells. The cytokines cause an inflammatory reaction by recruiting eosinophils, macrophages, neutrophils, and monocytes or natural killer cells, which my contribute to the inflammatory infiltrate. DTH reactions together with IgE – mediated mechanisms take part in the chronic eczematous reaction of atopic dermatitis, in humans, dogs and horses [3].

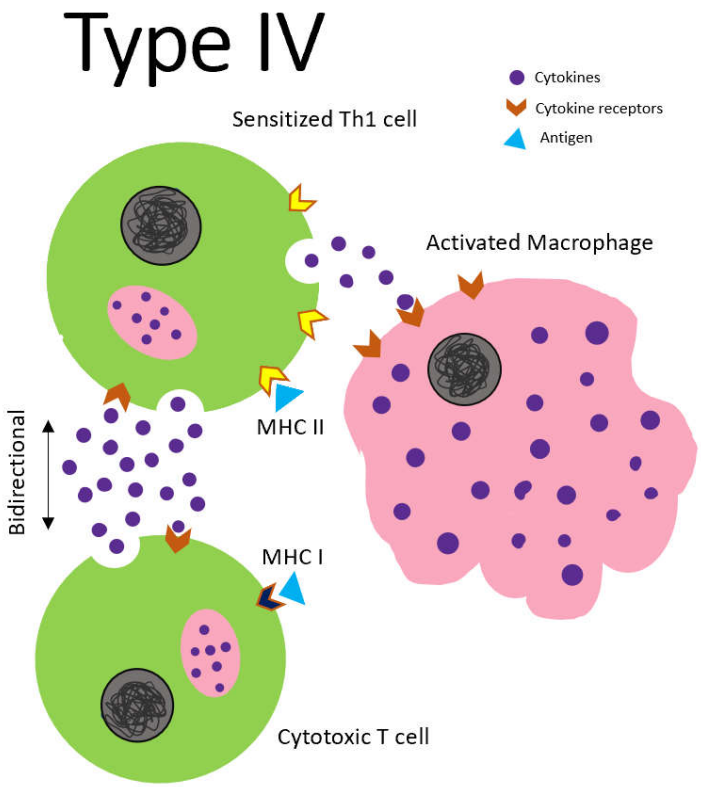

Figure 04: Scheme of type IV hypersensitivity reactions, adapted from [2].

1.2.5. Allergies in horses

Allergic reactions in horses are known for a long time [4-7]. Allergic horses may react to pollen with respiratory and skin symptoms, whereas less is known about food allergies in horses [8]. Studies have shown that there are several breeds, for example Dutch- and Swedish warmbloods, Oldenburgs, Icelandic horses and Paso finos, which have an higher risk for developing allergies [9, 10]. The symptoms of allergy related respiratory diseases range from immediate type reactions like coughing, rhinitis and asthma, to COPD (Chronic obstructive pulmonary disease) and emphysema as a consequence of chronic exposure [11]. Also delayed type hypersensitivity reaction can be observed in horses, RAO for example [10, 12].

1.2.6. Allergy testing in horses

Allergy testing in horses is most often done using intradermal testing and serum testing [9, 13]. Intradermal testing revealed pollen from Bermuda grass, weed, sage, olive, cedar, orange and alder as major respiratory allergens [13].

Depending on the clinical symptoms different diagnostic pathways can be followed. (Fig. 01).

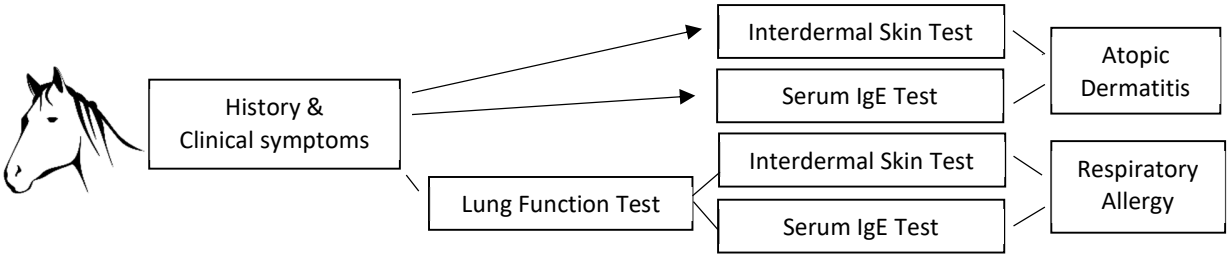

Figure 05: The diagnostic pathway to confirm pollen allergy in horses, adapted from [10].

1.3. Plants causing allergic reactions

Not all plants cause allergic reactions neither in humans nor animals. The reason is that plants have different forms of reproduction, affecting the geometric structure of their pollen. Gymnosperms are phylogenetically older, and their allergic potential is lower despite being pollinated by wind. Angiosperms, which are mostly pollinated by insects and other animals, but also by wind, are the most important elicitors of allergic reactions, and include pollen from trees, grasses or weeds [14].

Pollen are the most important aeroallergens and are studied well all across the world [15]. According to the seasons of the year plants can be clustered in four groups the early-, middle-, summer- and late bloomers.

1.3.1 Early bloomers

The early bloomers are the first plants pollinating in the new year, depending on the weather conditions, sometimes even in December. Important members of this category are *Alnus* (alder) flowering from December to June; *Corylus* (hazel) from January to April, Taxus (yew) from January to April; *Populus* (poplar) from February to April, *Ulmus* (elm) from February to May, *Salix* (willow) from February to June.

Alder, is the most important early bloomer relevant for horses [1]. Humans whereas react to hazel in this season, but cross reactions to other plants are possible [14].

1.3.2 Middle bloomers

After the early blooming plants, the middle bloomer season is following starting in March. Their important members are, *Carpinus* (hornbeam), *Fraxinus* (ash), *Ostrya* (hop hornbeam) and *Acer* (maple) from March to May; *Prunus* (cherry, Blackthorn), *Pinus* (pine) from March to June; *Betula* (birch) from April to May/June, *Platanus* (plane tree), *Juglans* (walnut), *Larix* (larch), *Fagus* (beech) *Abies* (Fir) from April to May; *Quercus* (oak), *Aesculus* (horse chestnut), from April to June.

For humans the most important middle bloomer is the birch tree, being overall the second most important allergen in the east of Austria. Alder, birch and hazel are members of the same family called *Betulaceae*, therefore IgE cross reactivity between those three are common in humans [14].

1.3.3 Summer bloomers / grass season

The summer bloomers, bloom in the so called grass season, and are the most important plants causing allergic reactions in humans [14, 16]. *Poaceae* (grasses) flower from May to September, but there are also other plants flowering during this time of year, like *Plantago* (plantain), *Rumex*

(dock/sorrel), from April to August; *Robinia* (robinia), *Tilia* (lime), *Ligustrum* (privet), *Sambucus* (elder) from May to June; *Castanea* (chestnut) from June to August; *Urticaceae* (nettle) from June to September.

Grass pollen allergies are widely spread in Europe, 8 % to 35 % of human adults are suffering from them [16]. For humans *Poa pratensis* (Kentucky bluegrass) is the most relevant grass in terms of allergy. For horses *Cynodon dactylon* (Bermuda grass) is a major allergen source [1]. Some plants, like grasses and nettles, with a flowering season lasting to September, they span the summer- to the late bloomer season.

1.3.4 Late bloomers

The late bloomers are the last flowering plants before winter. *Sophora* (pagoda tree), *Ailanthus* (tree of heaven) flower from July to August; *Solidago canadiensis* (Canadian golden rod) from July to October; *Artemisia* (mugwort) from July to September; *Ambrosia* (ragweed) from August to September; *Hedera* (ivy) from September to November. Also here, some plants flower over several season categories.

To humans ragweed is a highly allergenic plant and the distribution is increasing all over Europe [17]. Among the late bloomers only some of the horses were tested positive to Art v 3 and Art v 1 allergens from *Artemisia* (mugwort) [1].

1.3.5 Pollen distribution over the year

The distribution of pollen by a specific plant is dependent on the region and the local climate. **Figure 06** shows that the blooming seasons of trees, grasses and weeds in central Europe do partly overlap.

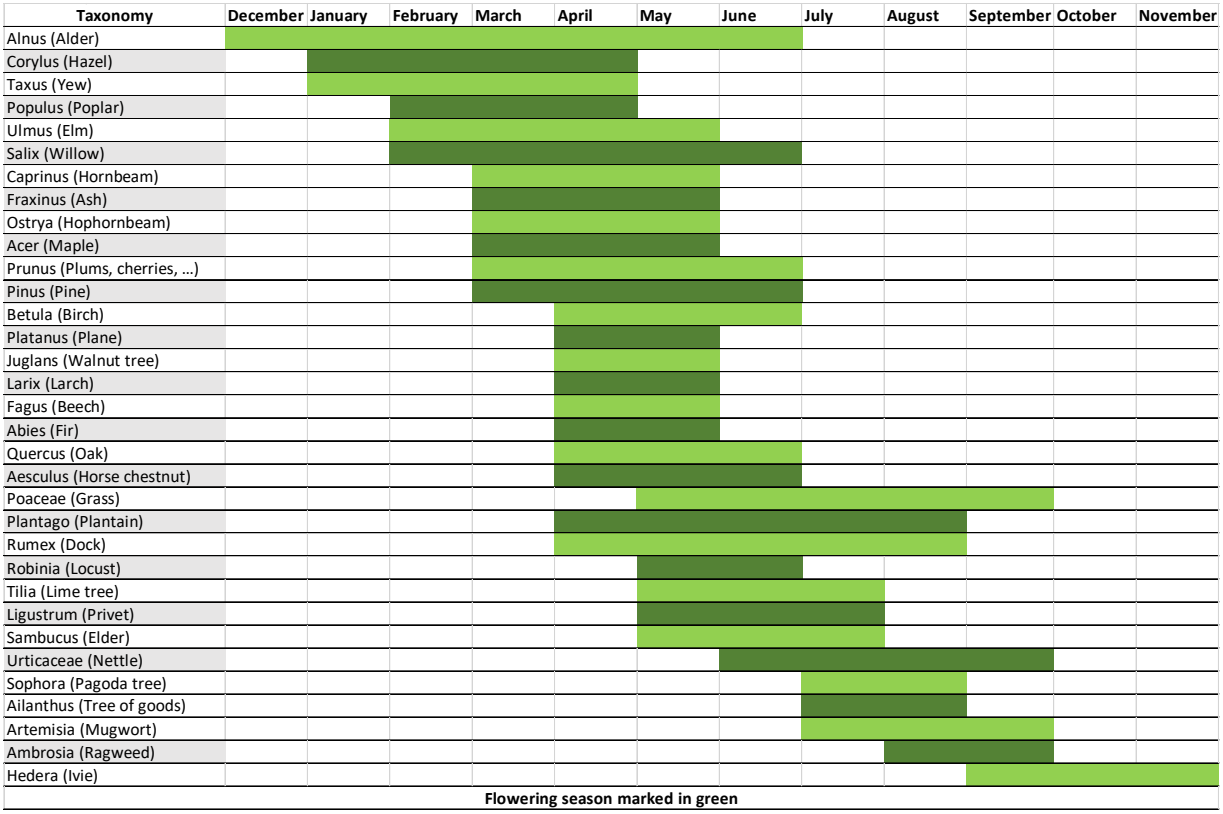

Figure 06: Plants and their allergen releasing periods distributed over the year [14, 16-19].© Korath

In Austria four periods with a particularly high pollen load, can be observed during a year. The pollen year starts in December and January with alder and hazel. In April the second "pollen wave" induced by ash and birch, followed by the grass season from May to July. Finally mugwort and ragweed have their flowering season in August till October [14].

1.3.6 Plants important for this study

The plants relevant for this study, which were found during the measurements at all testing locations, were (alphabetically) *Ailanthus* (tree of gods), *Alnus* (alder), *Amarantheceae* (amaranth family), *Ambrosia* (ragweed), *Betula* (birch), *Brassicaea* (crucifers), *Caryophyllaceae* (carnation family), *Cupressaceae* (cypress family), *Corylus* (hazel), *Fagus* (beech), *Fraxinus* (ash), *Juglans* (walnut tree), *Mercurialis* (mercuriies), *Morus* (mulberries), *Picea* (spruce), *Plantago* (plantains),

Poaceae (grasses), *Polygonaceae* (knotweed family), *Populus* (poplar, aspen, cottonwood), *Quercus* (oak), *Salix* (willow), *Sambucus* (elder) and *Urticaceae* (nettle family).

Allergenicity describes the potential of an allergen to sensitize and cause symptoms. It is determined by the intrinsic potency of a specific allergen. However, the abundances of an allergen in a certain environment determines exposure and hence, the risk of sensitization risk too.

In terms of allergenicity, *Betulaceae* like *Alnus*, *Betula* and *Corylus*, are highly important allergen sources for humans as well as to horses. The *Poaceae* a part of the *Gramineae* family have a high allergenicity, their flowering period lasts, species specifically, from May to September. *Asteraceae*, as *Ambrosia* or *Artemisa*, have a high allergenicity they are responsible for the "hay fever" in autumn. *Oleaceae* like *Fraxinus* they have a moderate allergenicity, but cross reactions are known with related plants. *Plantaginaceae*, such as *Plantago* have a moderate allergenic potential, mostly in combination with allergies against other grasses or herbs. *Salicaceae* like *Populus* and *Salix* are low in allergenicity, but there could be a mechanical irritation of the airways due to the size of their pollen. In addition, *Juglans* part of the *Juglandaceae*, as well as *Fagus* and *Quercus* members of the *Fagaceae* family and *Sambucus* a *Adoxaceae* are known to have a low allergenicity. *Polygonaceae*, like Rumex, too, but their allergenicity is often shadowed by a grass allergy. The in Europe widely spread *Urticaceae* also have a low potential to be an allergen. The tree of gods *Ailanthus*, member of the *Simaroubaceae* family is originated in Asia. After it was brought to Europe people contact hypersensitivity with this plant [14].

1.4. Aims of the study

The overall goal of the study was to answer why IgE profiles differ between humans and horses. We aimed thus to identify pollen exposure on horse grounds, in cooperation with the Austrian Pollen Service and to compare with the human urban exposure at the same timepoints. Further it was aimed to characterize plants on horse grounds phenologically in order to correlate the pollen exposure with plants growing locally at the testing locations. Furthermore, we intended to immunologically analyse the captured allergens. Finally, we expected to reveal whether the "Pollen App" by the Austrian Pollen Information Service is suitable for warning owners of allergic horses.

2. MATERIALS AND METHODS

2.1. Study design

The measurements were planned to include all flowering seasons therefore they had to be performed four times over 12 months. We started with the middle bloomer-, summer bloomer- and late bloomer season in 2018 and finished with the early bloomer - season of 2019.

2.1.1. Definitions

To verify the exposure levels on horse grounds, stables were selected which provide a pasture and a paddock for the horses. Paddocks were defined as an enclosure, where the horses spend their daytime standing on plane ground without any grass growing inside. Pastures were defined as areas, where the horses do grazing, with grass and plants covering the ground.

2.1.2. Study sides

For measuring, four different locations in lower Austria (A, B, C, D) within a radius of 45 kilometres around Vienna (centre) were chosen (Fig. 07). All these locations have paddocks and pastures for the horses, which provides us with in total eight points for measurements. All stables were inquired beforehand and written consent for measurements was given.

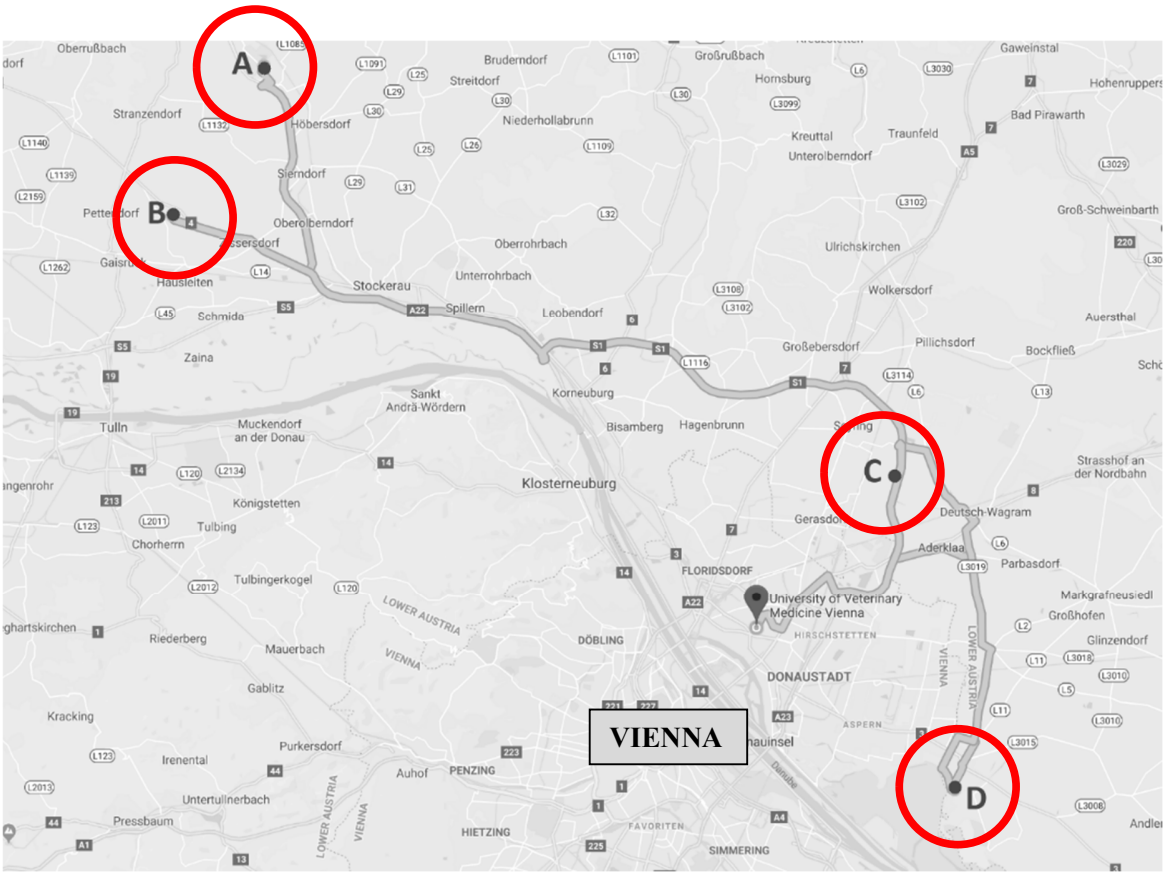

Figure 07: The maps show the testing locations. Map data © 2019 GeoBasis-DE/BKG (©2009), Google

2.1.3. Measuring dates

The study started in 2018 and was completed in 2019. The first measurement took place on April 25th 2018, and captured the flowering middle bloomer pollen. The second measurement was performed on June 1st 2018, representative for the grass flowering season. The 3rd measurement

was done on September 11th 2018 to measure the late bloomer pollen. The 4th and last measurement was done on February 28th 2019 and caught the early bloomer pollen.

2.1.4. Time points

Due to the limitation, that only two pollen traps were available, it was possible to measure on paddocks and pastures of one stable simultaneously, but not in all stables at the same time. The measurements took place following the order A to D all testing days.

2.2. Allergen detection

Two different devices were used to capture pollen for the quantitative and the qualitative measurements.

2.2.1 Pollen trap

Quantitative pollen measurements were done using a continuous recording air sampler, the Burkhart hirst-type volumetric spore trap (Burkhard Manufacturing Co. Limited, Hertfordshire, England). It was placed 28 cm above the ground and air was collected for one hour. The trap was loaded with a sticky microscopic slide covered with Vaseline to catch all particles carried by the air. Afterwards the slide was ejected, pollen were stained and fixed for transportation and analysis.

2.2.2 Air sampling

The Dustream collector® (Indoor Biotechnologies, Inc., Charlottesville, USA) designed to capture allergens indoors by attaching a filter device to a vacuum cleaner and collecting allergens from a surface, e.g. a mattress. In this study instead of cleaning a surface we “collected” air by turning the vacuum cleaner with the Dustream collector® towards the wind direction. In this study a Dyson 32DC vacuum cleaner, which an airflow of 28 litres per second, was used. The sample filters were stored in 50 ml falcons at -20 °C until extraction and further analysis.

2.3. Botanical characterisation

During the air sampling times, the surroundings were examined phenologically and the occurring plants and their flowering status determined. The plant characterisations were done by our collaborating experts from the Austrian Pollen Information Service.

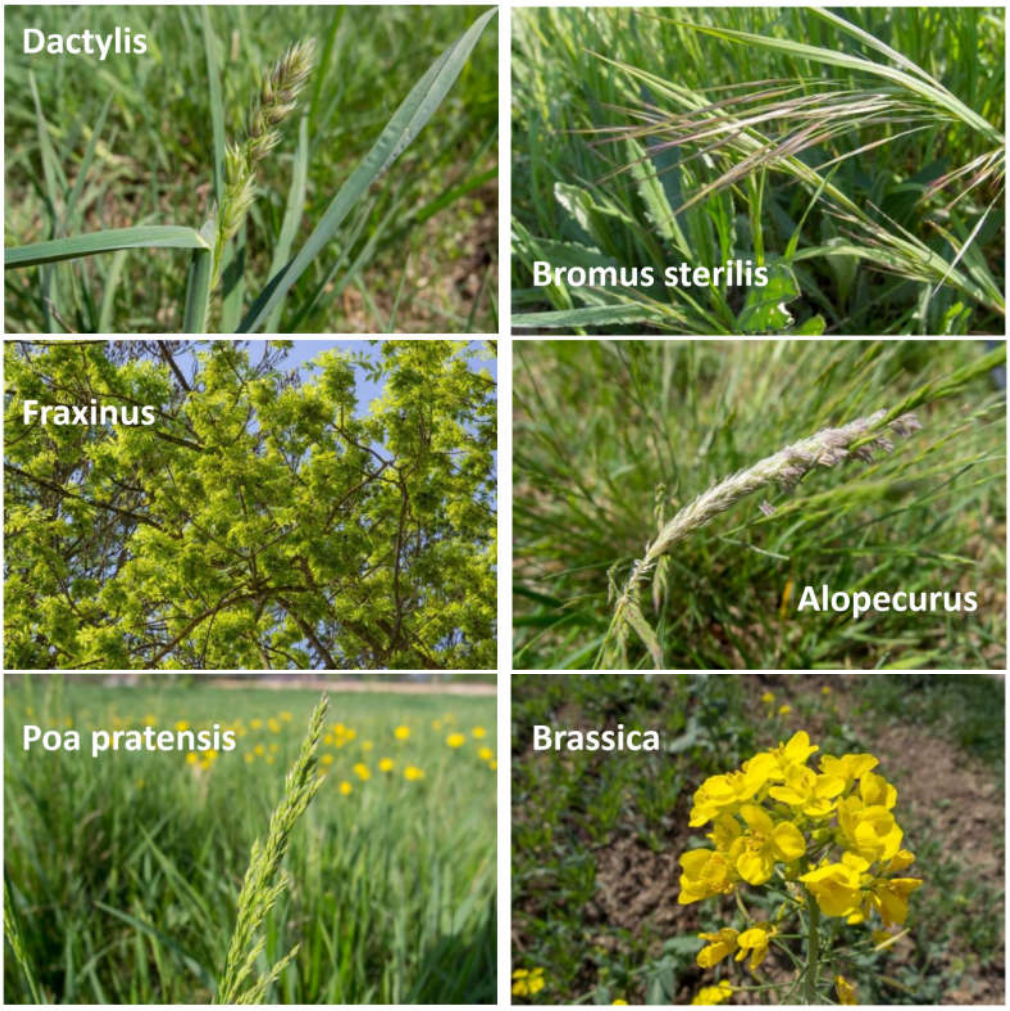

Figure 08: Examples of allergenic plants in horse areas. © Katharina Bastl

2.4. Quantitative pollen analyses

After pollen sampling, the captured allergens were stained alkalic fuchsin and fixed to the microscope slide using a mixture of Mowiol® a polyvinyl alcohol solved in ddH_2O with glycerine. Counting was done using a standard sampling procedure.

2.5. Qualitative pollen analyses

2.5.1 Extraction

To extract the pollen from the filter, the protocol by Buters et al. 2008 was slightly modified by replacing BSA (bovine serum albumin) with Trehalose, to avoid albumin interference but protecting the proteins during lyophilisation [20, 21].

Material:

Extraction buffer:

0.1 mol	Ammonium bicarbonate (A6141, SIGMA-ALDRICH™ Inc., St. Louis, USA)
solved in ddH_2O	
pH 8.1 aligned with 25 % Ammonia (CL00.0115, CHEM-LAB NV, Zedelgem, Belgium)	

2 ml extraction buffer per mg sample were added to the 50 ml falcon with the sample filter inside and incubated 4 hours in the dark on the shaker (ROLLER 6 digital, IKA, Staufen, Germany) with 60 rpm (rounds per minute). The filter was then transferred into another 50 ml falcon and both falcons were spun down 10 min per 3200 g to separate the liquid from the filter. The liquids were combined, split in 2.5 ml aliquots and stored at -80 °C.

2.5.2 Lyophilisation

The caps of the frozen samples were removed and replaced with perforated ones and samples were placed in a lyophiliser (Alpha 1-2 LDplus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) for two days. The main drying program was used, setting a vacuum

with the temperature set to -20 °C and 1 millibar pressure. After lyophilization caps of tubes were immediately replaced with closed ones and the samples stored at -20 °C.

2.5.3 Determination of the protein concentration

For determining the protein content, a photometric spectral analysis was done using the NanoPhotometer® P 300 (Implen GmbH, Munich, Germany). The function Absorbance Ration was used measuring at three different wavelengths, 260 nano meter (nm) and 280 nm as measuring points, while 320 nm was set as background. The extraction buffer, without a sample, was used as blank. The dilution factor was calculated by the nanophotometer and the results of the concentration of each sample was given in µg / µl.

2.5.4 Immunological characterisation

2.5.5.1 SDS – PAGE (Sodium dodecyl sulphate–polyacrylamide gel electrophoresis)

Material:

Recombinant Bet v 1 a = 0.17 mg / ml

Recombinant Phl p 5 a = 40µg / ml

Marker: Page Ruler® (26616, Thermo Fisher Scientific, Massachusetts, USA)

4-20% Mini-PROTEAN® TGX Stain-free Gels (Bio-Rad Laboratories Ges.m.b.H., Wien, Austria)

To separate the proteins according to their molecular masses an SDS PAGE was performed. To achieve an optimal separation, gradient gel 4 - 20% Mini-PROTEAN® TGX Stain-free Gels (Bio-Rad Laboratories Ges.m.b.H., Wien, Austria). The concentration was adjusted to the lowest sample.

Electrophoresis: 12 µl samples or controls were incubated with 4 µl sample buffer for 5 minutes (min) at 95 °C on BioShake IQ® shaker (BioShake IO®, Quantifoil Instruments GmbH, Jena, Germany). After a fast spin down the gel was loaded with 10 µl of each sample and run with 140 Volt for 60 min (PowerPac Basic, Bio-Rad Laboratories Ges.m.b.H., Wien, Austria).

2.5.5.2 Silver staining

For visualisation of the proteins after SDS PAGE, silver staining was performed.

Materials:

Gel fixing solution:

50 ml	Ethanol (603-002-00-5, Merck KGaA, Darmstadt, Germany)
10 ml	Acetic Acid (20103-295, VWR International, Pennsylvania, USA)
solved in 40 ml ddH_2O	

Washing solution:

30 ml	Ethanol (603-002-00-5, Merck KGaA, Darmstadt, Germany)
solved in 70 ml ddH_2O	

Thiosulfate reagent:

100 mg	Sodium thiosulfate (0,02%) (S1648, SIGMA-ALDRICH™ Inc., St. Louis, USA)
solved in 500 ml ddH_2O	

Silver nitrate reagent:

1 g	Silver nitrate (0,2%) (S6506, SIGMA-ALDRICH™ Inc., St. Louis, USA)
100 µl	(37%) Formaldehyde (0,02%) (4003, Merck KGaA, Darmstadt, Germany)
solved in 500 ml ddH_2O – stored under exclusion of light	

Developer:

15 g	Sodium carbonate (6392, Merck KGaA, Darmstadt, Germany)
250 µl	(37%) Formaldehyde (0,05 %) (4003, Merck KGaA, Darmstadt, Germany)
2,5 mg	Sodium thiosulfate (S1648, SIGMA-ALDRICH™ Inc., St. Louis, USA)
solved in 500 ml ddH_2O – stored 4 °C	

Stop solution:

2,5 g	Glycine (0,5%) (1154KG005, BioFroxx GmbH, Einhausen, Germany)
solved in 500 ml ddH_2O – stored 4 °C	

After running the SDS PAGE the gel was incubated in fixing solution for 1 hour room temperature (RT) or at 4 °C over night. As the first of the silver staining the gel was washed with ddH_2O three times for 20 min. Then it was incubated in Thiosulfate reagent for 1 min, followed by three times washing with ddH_2O for 20 sec. The gel was then covered with silver nitrate reagent and incubated for 20 min, then the gel was rinsed with ddH_2O and developer solution added. When the proteins were proper visible, the developer was discarded and the gel was incubated in stop solution for 5 min.

To visualize the results of the silver stain, the imaging system ChemiDoc™ Touch (Bio-rad Laboratories Ges.m.b.H., Vienna, Austria) was used. The gels were run on the white plate for transillumination of colorimetric gels, and the program "protein gel" with "silver stain", automatic mode with high resolution, was used.

2.5.5.3 Western blot

2.5.5.4.1. Electro blotting

To perform the immunological characterization the proteins were separated according to their molecular mass and transferred from the gel on to a nitrocellulose membrane, performing an electroblot.

Materials:

Transfer buffer:

10x Transfer buffer:	
30.0 g	Trixme Base (250 M) (T1503, SIGMA-ALDRICH™ Inc., St. Louis, USA)
145 g	Glycine (1.92 M) (1154KG005, BioFroxx GmbH, Einhausen, Germany)
solved in 1000 ml ddH_2O	

1x Transfer buffer	
100 ml	10x Transfer buffer (250 M)
200 ml	Methanol (20%) (32213/CL01307, SIGMA-ALDRICH™ Inc., St. Louis, USA)
solved in 1000 ml ddH_2O	

For the transfer we used Amersham Protran 0.2 nitrocellulose 300MMX4M membrane (GE Healthcare Europe GMBH, Vienna, Austria), which was preincubated in 1x transfer buffer. After the SDS PAGE the gel was incubated in transfer buffer for 10 min. Also, all parts of the sandwich were soaked in transfer buffer. To assemble the semi-dry sandwich, six layers of wipers were put in the middle of the lower part of the cassette, then the nitrocellulose membrane was put on top of it. Followed by the gel and another six layers of wipers (Fig. 09).

Top of the cassette
6 layers of wipers
Gel
Nitrocellulose membrane
6 layers of wipers
Bottom of the cassette

Figure 09: Scheme of the semi-dry sandwich assembly

After the assembly the cassette was closed properly and inserted in the Trans -Blot® Turbo™ (Bio-Rad Laboratories Ges.m.b.H., Wien, Austria). To transfer the pre-existing program "StandardSD MINI", for mini gels, with 25 Volt 1.0 Ampere for 30 min was run.

2.5.5.4.2. Immuno blotting

After the transfer to nitrocellulose the immunoblot was performed with a horse raddish peroxide (HRP)-labelled anti-Bet v 1-human-IgG_1, to determine if the specific birch pollen protein Bet v 1 is on the membrane.

Materials:

10 x Phosphate Buffered Saline (PBS):

80 g	Sodium Chloride 1.36 M (31414, SIGMA-ALDRICH™ Inc., St. Louis, USA)
2.4 g	Monopotassium phosphate 18 mM (4360534, VWR International, Pennsylvania, USA)
2.0 g	Potassium Chloride 27 mM (104938, Merck KGaA, Darmstadt, Germany)
14.2 g	Sodium Hydrogen phosphate 100 mM (71642, SIGMA-ALDRICH™ Inc., St. Louis, USA)
solved in 1000 ml ddH_2O	

Washing buffer: PBS-T 0.1 %

20 ml	10x PBS
0.2 ml	Tween (T) 20
solved in 200 ml ddH_2O	

Blocking buffer: PBST 0.1 % with 5 % BSA

20 ml	PBS-T 0.1 %
1 g	Bovine Serum Albumin 5 % (P1379/0777, SIGMA-ALDRICH™ Inc., St. Louis, USA)

Dilution buffer: PBST 0.1 % with 0.5 % BSA

20 ml	PBS-T 0.1 %
0.1 g	Bovine Serum Albumin 0.5 % (P1379/0777, SIGMA-ALDRICH™ Inc., St. Louis, USA)

First Antibody: human Anti-Bet v 1 IgG_1 in house antibody (M0418) (ms in prep. Koehler et al.)

Secondary Antibody: mouse Anti-human IgG_1 Fc-HRP (SouthernBiotech, Birmingham, USA)

Clarity® Western ECL substrate (170-5061, Bio-Rad Laboratories, Inc., California, USA

First the membrane was blocked with 5 % bovine serum albumin (BSA) solved in PBST 0.1% incubated for 2 hours at RT, followed by 3 washing steps for 10 min with ddH_2O. The membrane was then incubated for 1 hour RT with the anti-birch human IgG_1. After washing the membrane 3 times for 10 min with ddH_20, the membrane was incubated for 1 hour with the HRP-labelled anti-

human IgG_1 secondary antibody, diluted 1:10000. After washing the membrane 3 times for 10 min with H_20dd, ECL substrate was applied to develop bound anti-human IgG_1 antibodies.

To visualize the results of the Western blot, the imaging system ChemiDoc™ Touch (Bio-rad Laboratories Ges.m.b.H., Vienna, Austria) was used. The gels were run on the black sample tray for imaging chemiluminescence and the program blots with Chemiluminescence on Rapid automatic exposure mode was used. To make the Marker visible an additional picture was taken using the Ethidiumbromide program. Afterwards the two pictures were combined into a composite picture, who show the marker and the results of the Western blot.

3. RESULTS

3.1 Quantitative analyses of pollen exposure

The analyses of the four testing locations A – D consisted of the phenological analyses of plants and the determination of pollen exposure levels.

3.1.1 Botanical characterisation of plants at horse grounds

During each measurement, an expert assessed the surroundings phenologically, to correlate the flora composition with the simultaneously captured pollen. The analyses revealed that there are local differences in the botanical environment of the four testing locations.

In the **early bloomer season** revealed that at location A and B flowering *Corylus avellana* were observed, while locations C and D had no local early blooming plants around on horse grounds, neither paddocks nor pastures.

In the **middle bloomer season** flowering *Acer* was observed at location A, C and faded at location D. Flowering *Aesculus* was found at location A. Flowering *Asteraceae* were detected at the locations A, B and C. At location A and B flowering *Betula* was observed. Nearby fields had flowering *Brassica napus* at the locations A and B. Faded *Fraxinus* was located at stable A. *Juglans* was found flowering at location B and faded at D. A *Pinus*, which was not net flowering was found at stable D. Flowering *Plantago* could be seen at the stables B and C. At the locations C and D faded *Populus* was found. The stable D also had flowering *Ranunculus* nearby. Not yet flowering *Robinia* could be seen at stable C. Flowering *Rosaceae* were found at B and D. At location A faded *Salix* was detected and at stable C a flowering *Syringa*. In addition, several *Poaceae* were found at the locations, flowering *Alopecurus* at stable B and C. Not flowering *Bromus sterilis* at A, B and C, *Dactylis glomerate* at stable D, *Festuca angustifolia* at location C and *Poa pratensis* at all four locations.

Of course, lots of *Poaceae* were found during the **grass season**. *Alopecurus* was observed faded at location A and B. At location C faded *Arrhenatherum elatius* was found. Flowering *Bromus inermis* was detected at stable A and in a very high amount at location C. Location C and D had fading *Dactylis glomerata*, A and B had some still flowering. *Elymus* was found flowering at B, C and in a high amount at A. The *Festuca angustifolia* were found faded at location C and flowering at B. Stable A had flowering *Festuca pratensis*. Already faded *Hordeum murinum* was found at location A, B and C. All four stables had *Lolium perenne* nearby, at B, C, D flowering and at A only partly flowering. Faded *Poa pratensis* was also observed at all four locations. In Stable B flowering *Trisetum flavescens* was seen. It is needed to be noticed, that at location D had been mowed in large-scale. On the fields around the testing locations, faded *Triticum* was found at location A and not yet flowering *Hordeum vulgare* was detected at stable B. During the grass season also following plants were found, flowering *Apiaceae* and not flowering *Artemisia* were found at location A and C. Faded *Asteraceae* could be detected at stable A and D. *Chenopodiaceae* were starting to flower at location B. *Plantago lanceolate* were observed flowering at all four locations at D there were also *Plantago media*. Flowering *Ranunculus* was seen at location B. At stable C *Robinia* was found already faded. At A and C a variety of *Urticaceae* was flowering.

In the **late bloomer season** only at stable D a flowering *Ambrosia artemisiifolia* was found. *Apiaceae* were detected flowering at A, D and faded at location C. Already faded *Artemisia* were observed at the location A and C. Several different *Asteraceae* were detected, some flowering, some faded at stable D, flowering at stable B and C. At all four locations *Chenopodiaceae* were found, flowering at A, B, D and mostly faded just a few flowering at location C. *Hedera* at the begin of flowering was found at location A. *Plantago lanceolata* was found flowering at stable A, faded at C, mostly faded, a few flowering at D and at B they found it flowering with *Plantago media* faded. Also flowering *Solidago canadiensis* was seen at location A and B. *Trifolium* was found flowering at stable D. Several *Urticaceae* were found flowering at stable A and B. During that measurement several *Poaceae* were detected. *Cynodon dactylon* was seen flowering at stable A and mostly faded with some flowering at stable D. Faded *Phalaris* sp. were seen at location A.

At all four locations *Setaria* sp. were observed faded. And faded *Zea mays* could be seen at stable B. Again, it must be noticed, that at location D had been mowed in large-scale.

Taken together flowerings at stables A – D were highly variable in all seasons and characteristic for each specific local environment.

3.1.2 Counts of the captured pollen

3.1.2.1 Overview

During the quantitative measurements a variety of pollen was found. In the cycle of one year we found pollen from (alphabetically) *Abies*, *Acer*, *Aesculus*, *Ailanthus*, *Alnus*, *Amaranthaceae*, *Ambrosia*, *Apiaceae*, *Artemisia*, *Asteraceae*, *Betula*, *Brassicaceae*, *Cannabaceae*, *Carpinus*, *Caryophyllaceae*, *Castanea*, *Centaurea*, *Corylus*, *Cupressaceae*, *Cyperaceae*, *Fabaceae*, *Fagus*, *Fraxinus*, *Ginkgo*, *Humulus*, *Impatiens*, *Indet*, *Juglans*, *Ligustrum*, *Mercurialis*, *Morus*, *Ostrya*, *Phacelia*, *Philadelphus*, *Picea*, *Pinus*, *Plantago*, *Platanus*, *Poaceae*, *Polygonaceae*, *Populus*, *Quercus*, *Ranunculaceae*, *Rosaceae*, *Rubiaceae*, *Rumex*, *Salix*, *Sambucus*, *Secale*, *Syringa*, *Tamarix*, *Tilia*, *Triticum*, *Typhaceae*, *Ulmus*, *Urticaceae*, *Xantium*, *Zea Mays*. The comparison between pasture and paddock over all seasons are shown in the **Tables 01 – 04**.

Seasonal differences also were observed in the captured pollen. The majority of *Alnus, Corylus, Cupressaceae* pollen were found in February 2019 because they are early blooming plants. Pollen of the middle bloomers, *Betula*, *Brassicaceae*, *Caryophyllaceae*, *Fagus*, *Fraxinus*, *Juglans*, *Morus*, *Picea*, *Platanus*, *Poaceae*, *Populus*, *Quercus*, *Rosaceae*, *Salix*, *Syringa*, *Tamarix*, were mostly found in April 2018. In June 2018 the measurement during the grass season predominantly *Ailanthus*, *Asteraceae*, *Centaurea*, *Pinus*, *Plantago*, *Poaceae*, *Sambucus*, *Tilia*, *Urticaceae* were found. The late bloomer measurement in September 2018 *Amaranthaceae*, *Ambrosia*, *Artemisia*, *Asteraceae*, *Castanea*, *Fabaceae*, *Humulus*, *Indet*, *Mercurialis*, *Phacelia*, *Plantago*, *Poaceae*, *Polygonaceae*, *Rumex*, *Zea Mays* were found.

Overall the highest pollen count was found in the early bloomer season and the least amount of pollen was captured in the late bloomer season. Interestingly, at most of the times the pollen exposure on the paddocks was higher compared to the pastures.

Table 01: Captured pollen in the early bloomer season. A-D…location; VIE… Vienna same time as stables; 1…pasture, 2….paddock; low counts < 10 grains / m³, middle counts 10 – 100 grains / m³ high counts < 100 grains / m³

early		A			B			C			D	
bloomers	1	2	VIE	1	2	VIE	1	2	VIE	1	2	VIE
Abies	0	0	0	0	0	0	0	0	0	0	0	0
Acer	0	0	0	0	0	0	0	1	0	0	0	0
Aesculus	0	0	0	0	1	0	0	0	0	0	0	0
Ailanthus	0	0	0	0	0	0	0	0	0	0	0	0
Alnus	69	133	0	121	152	115	79	307	91	198	293	116
Amaranthaceae	0	0	1	0	2	0	0	0	0	0	0	0
Ambrosia	0	0	1	0	0	0	0	0	0	0	0	0
Apiaceae	0	0	0	0	0	0	0	0	0	0	0	0
Artemisia	0	0	0	0	0	0	0	0	0	0	0	0
Asteraceae	0	0	0	0	0	0	0	0	0	0	0	0
Betula	0	0	0	0	0	0	0	0	0	0	0	0
Brassicaceae	0	0	0	0	0	0	0	0	0	0	0	0
Cannabaceae	0	0	0	0	0	0	0	0	0	0	0	0
Carpinus	0	0	0	0	0	0	0	0	0	0	0	0
Caryophyllaceae	0	0	0	0	0	0	0	0	0	0	0	0
Castanea	0	0	0	0	0	0	0	0	0	0	0	0
Centaurea	0	0	0	0	0	0	0	0	0	0	0	0
Corylus	21	38	0	27	45	12	20	66	19	41	79	28
Cupressaceae	13	21	0	169	206	973	7	11	743	310	499	223
Cyperaceae	0	0	0	0	0	0	0	0	0	0	0	0
Fabaceae	0	0	0	0	0	0	0	0	0	0	1	0
Fagus	0	1	0	0	0	0	0	0	0	0	0	0
Fraxinus	0	0	0	0	0	0	0	0	0	0	2	0
Ginkgo	0	0	0	0	0	0	0	0	0	0	0	0
Humulus	0	0	0	0	0	0	0	0	0	0	0	0
Impatiens	0	0	0	0	0	0	0	0	0	0	0	0
Indet	0	0	0	0	0	0	0	0	0	1	0	0
Juglans	1	0	0	0	0	0	0	0	0	1	0	0
Ligustrum	0	0	0	0	0	0	0	0	0	0	0	0
Mercurialis	0	0	0	0	0	0	0	0	0	0	0	0
Morus	0	0	0	0	0	0	0	0	0	0	0	0
Ostrya	0	0	0	0	0	0	0	0	0	0	0	0
Phacelia	0	0	0	0	0	0	0	0	0	0	0	0
Philadelphus	0	0	0	0	0	0	0	0	0	0	0	0
Picea	0	2	0	1	0	1	1	0	1	1	1	0
Pinus	0	0	0	0	0	0	0	0	0	0	1	0
Plantago	1	0	0	0	0	0	1	1	0	0	0	0
Platanus	0	0	0	0	0	0	0	0	0	0	0	0
Poaceae	0	4	2	0	1	0	3	0	0	0	0	0
Polygonaceae	0	0	0	0	0	0	0	1	0	0	0	0
Populus	1	2	0	1	2	1	1	2	0	3	17	1
Quercus	0	0	0	0	0	0	0	0	0	0	0	0
Ranunculaceae	0	0	0	0	0	0	0	0	0	0	0	0
Rosaceae	0	0	1	0	0	0	0	0	0	0	0	0
Rubiaceae	0	0	0	0	0	0	0	0	0	0	0	0
Rumex	0	0	0	0	0	0	0	0	0	0	0	0
Salix	0	0	0	0	0	0	0	0	0	0	1	0
Sambucus	0	0	0	0	0	0	0	0	0	0	0	0
Secale	0	0	0	0	0	0	0	0	0	0	0	0
Syringa	0	0	0	0	0	0	0	0	0	0	0	0
Tamarix	0	0	0	0	0	0	0	0	0	0	0	0
Tilia	0	0	0	0	0	0	0	0	0	1	0	0
Triticum	0	0	0	0	0	0	0	0	0	0	0	0
Typhaceae	0	0	0	0	0	0	0	0	0	0	0	0
Ulmus	0	0	0	0	0	1	0	0	1	1	3	1
Urticaceae	0	0	3	0	0	0	0	0	0	0	0	0
Xantium	0	0	0	0	0	0	0	0	0	0	0	0
Zea Mays	0	0	0	0	0	0	0	0	0	0	0	0

Table 02: Captured pollen in the middle bloomer season. A-D…location; VIE… Vienna same time as stables; 1…pasture, 2….paddock; low counts < 10 grains / m³, middle counts 10 – 100 grains / m³ high counts < 100 grains / m³

middle		A			B			C			D	
bloomers	1	2	VIE	1	2	VIE	1	2	VIE	1	2	VIE
Abies	0	1	1	1	1	1	1	1	0	3	1	0
Acer	0	0	0	2	2	0	0	1	0	1	0	0
Aesculus	0	0	3	0	0	7	0	2	7	3	4	5
Ailanthus	0	0	0	0	0	0	0	0	0	0	0	0
Alnus	0	0		2	3		0	4	1	0	5	1
Amaranthaceae	0	0	0	0	0	0	0	0	0	0	0	0
Ambrosia	0	0	0	0	0	0	0	0	0	0	0	0
Apiaceae	0	0	0	0	1	0	0	0	0	0	1	0
Artemisia	0	0	0	0	0	0	0	0	0	0	0	0
Asteraceae	0	0	0	9	0	0	0	1	0	1	1	0
Betula	0	5	6	51	23	16	6	37	20	16	49	16
Brassicaceae	0	3	0	13	8	0	0	0	0	6	0	0
Cannabaceae	0	0	0	0	0	0	0	0	0	0	0	0
Carpinus	1	0	2	6	4	4	0	9	4	2	3	1
Caryophyllaceae	0	0	0	13	0	0	0	0	0	0	0	0
Castanea	0	0	0	0	0	0	0	0	0	0	0	0
Centaurea	0	0	0	0	0	0	0	0	0	0	0	
Corylus	0	0	0	1	0	0	0	1	1	0	1	1
Cupressaceae	0	0		16	4		0	8	48	1	3	12
Cyperaceae	0	0	0	1	1	0	0	1	1	0	1	1
Fabaceae	0	0	0	0	0	0	0	0	0	0	0	0
Fagus	0	3	7	77	44	34	4	33	40	37	79	19
Fraxinus	0	6	20	17	8	14	2	12	6	5	8	3
Ginkgo	0	0	0	0	0	0	0	0	0	0	1	0
Humulus	0	0	0	0	0	0	0	0	0	0	0	0
Impatiens	0	0	0	0	0	0	0	0	0	0	0	0
Indet	0	0	0	3	0	0	0	0	0	0	2	0
Juglans	0	0	10	16	8	16	5	11	10	8	30	7
Ligustrum	0	0	0	0	0	0	0	0	0	0	0	0
Mercurialis	0	0	0	0	0	0	0	0	0	0	0	0
Morus	0	0	19	11	9	42	0	0	45	23	55	30
Ostrya	0	0	0	1	0	1	0	1	1	1	2	1
Phacelia	0	0	0	0	0	0	0	0	0	0	0	0
Philadelphus	0	0	0	0	0	0	0	0	0	0	0	0
Picea	1	2	8	59	28	26	42	121	49	95	228	53
Pinus	0	0	5	2	2	13	3	6	10	2	6	5
Plantago	0	1	0	4	0	0	0	2	0	1	2	1
Platanus	0	2	6	4	7	6	2	8	6	3	6	4
Poaceae	0	0	1	1	4	1	0	8	0	0	9	1
Polygonaceae	0	0	0	0	0	0	0	0	0	0	0	0
Populus	0	0	1	5	6	1	1	0	1	0	2	0
Quercus	0	13	33	90	74	60	7	82	46	33	121	30
Ranunculaceae	0	0	1	0	0	2	0	0	2	0	0	0
Rosaceae	0	0	2	2	3	2	1	3	4	0	1	3
Rubiaceae	0	1	0	0	0	0	0	0	0	0	0	0
Rumex	0	0	1	1	1	1	0	0	0	0	1	1
Salix	1	3	3	14	10	3	0	5	3	2	7	3
Sambucus	0	0	0	0	0	0	0	0	0	0	0	0
Secale	0	0	0	0	0	0	0	0	0	0	0	0
Syringa	0	0	0	4	2	0	0	0	0	0	0	0
Tamarix	0	0	3	1	1	2	0	1	2	0	1	2
Tilia	0	0	0	0	0	0	0	0	0	0	0	0
Triticum	0	0	0	0	0	0	0	0	0	0	0	0
Typhaceae	0	0	0	0	0	0	0	0	0	0	0	0
Ulmus	0	0	0	1	0	0	0	0	0	0	0	0
Urticaceae	0	0	0	1	0	0	0	0	0	0	0	0
Xantium	0	0	0	0	0	0	0	0	0	0	0	0
Zea Mays	0	0	0	0	0	0	0	0	0	0	0	0

Table 03: Captured pollen in the summer bloomer season. A-D…location; VIE… Vienna same time as stables; 1…pasture, 2….paddock; low counts < 10 grains / m³, middle counts 10 – 100 grains / m³ high counts < 100 grains / m³

summer		A			B			C			D	
bloomers	1	2	VIE	1	2	VIE	1	2	VIE	1	2	VIE
Abies	0	0	0	0	0	0	0	0	0	0	0	0
Acer	0	0	0	0	0	0	0	0	0	0	0	0
Aesculus	0	0	0	0	0	0	0	0	0	0	0	0
Ailanthus	3	0	1	3	12	2	1	1	2	3	4	2
Alnus	1	1	0	0	1	0	1	2	0	0	2	0
Amaranthaceae	0	0	0	0	0	0	0	1	0	1	1	0
Ambrosia	0	0	0	0	0	0	0	0	0	0	0	0
Apiaceae	0	1	0	0	0	0	2	0	0	0	0	0
Artemisia	0	0	0	0	0	0	0	0	0	0	0	0
Asteraceae	0	1	0	0	1	0	0	0	0	1	4	0
Betula	0	1	0	1	0	0	3	6	0	3	4	0
Brassicaceae	0	0	0	0	0	0	0	0	0	1	0	0
Cannabaceae	1	0	0	0	0	0	0	2	0	0	0	0
Carpinus	0	1	0	3	0	0	0	0	1	1	0	0
Caryophyllaceae	0	0	0	0	0	0	0	0	0	0	0	0
Castanea	2	0	0	0	3	0	0	0	1	0	0	1
Centaurea	2	0	0	0	0	0	0	0	0	1	1	0
Corylus	0	0	0	0	0	0	0	0	0	0	0	0
Cupressaceae	0	0	1	1	0	0	1	1	0	0	0	0
Cyperaceae	1	0	0	0	0	0	0	0	0	0	0	0
Fabaceae	0	0	0	0	0	0	0	0	0	0	0	0
Fagus	1	0	0	0	0	0	1	1	0	0	1	0
Fraxinus	0	0	0	0	0	0	1	0	0	0	0	0
Ginkgo	0	0	0	0	0	0	0	0	0	0	0	0
Humulus	0	0	0	0	0	0	0	0	0	0	0	0
Impatiens	0	0	0	0	0	0	0	0	0	0	0	0
Indet	5	3	0	2	1	0	2	3	0	1	2	0
Juglans	1	2	0	0	0	0	0	0	0	0	0	0
Ligustrum	0	0	0	2	4	0	0	1	0	1	0	0
Mercurialis	0	0	0	0	0	0	2	1	0	0	0	0
Morus	1	0	0	0	0	0	0	0	0	0	0	0
Ostrya	0	0	0	0	0	0	0	0	0	0	0	0
Phacelia	0	0	0	0	1	0	0	0	0	0	0	0
Philadelphus	0	0	0	0	0	0	0	1	0	0	0	1
Picea	4	5	1	3	0	2	1	2	0	1	3	
Pinus	1	1	2	4	4	1	5	2	0	0	10	1
Plantago	23	8	4	54	4	4	9	5	3	12	14	1
Platanus	1	0	0	0	0	0	1	0	0	0	0	0
Poaceae	88	55	21	84	71	26	362	111	14	23	82	10
Polygonaceae	0	0	0	0	0		0	0		0	0	0
Populus	0	0	0	0	0	0	0	0	0	0	0	0
Quercus	1	1	0	2	0	0	4	2	1	3	6	1
Ranunculaceae	0	0	0	0	1	0	1	0	0	0	0	0
Rosaceae	0	0	1	1	1	1	1	1	0	2	3	0
Rubiaceae	6	7	1	0	1	1	0	1	1	0	0	0
Rumex	0	2	0	0	2	0	0	1	0	0	1	0
Salix	0	1	1	0	0	1	1	0	0	1	1	0
Sambucus	6	22	1	7	5	2	4	10	0	8	7	0
Secale	0	1	0	1	0	0	0	0	0	0	0	0
Syringa	0	0	0	0	0	0	0	0	0	0	0	0
Tamarix	0	0	0	0	0	0	0	0	0	0	0	0
Tilia	4	0	7	4	1	12	0	4	9	2	6	2
Triticum	0	1	0	0	0	0	1	2	0	0	0	0
Typhaceae	0	0	1	6	0	0	2	1	0	1	0	0
Ulmus	0	0	0	0	0	0	0	0	0	0	0	0
Urticaceae	43	283	3	10	25	4	17	37	4	61	28	8
Xantium	0	0	0	0	0	0	0	0	0	0	0	0
Zea Mays	0	0	0	0	0	0	0	0	0	0	0	0

Table 04: Captured pollen in the late bloomer season. A-D…location; VIE… Vienna same time as stables; 1…pasture, 2….paddock; low counts < 10 grains / m³, middle counts 10 – 100 grains / m³ high counts < 100 grains / m³

late		A			B			C			D	
bloomers	1	2	VIE	1	2	VIE	1	2	VIE	1	2	VIE
Abies	0	0	0	0	0	0	0	0	0	0	0	0
Acer	0	0	0	0	0	0	0	0	0	0	0	0
Aesculus	0	0	0	0	0	0	0	0	0	0	0	0
Ailanthus	0	0	0	0	0	0	0	0	0	0	0	0
Alnus	0	0	0	0	0	0	0	0	0	0	1	0
Amaranthaceae	1	12	1	7	15	2	9	10	0	8	6	0
Ambrosia	3	4	1	2	2	0	2	2	0	0	2	0
Apiaceae	0	0	0	0	0	0	0	0	0	0	0	0
Artemisia	1	1	0	1	3	0	0	1	0	0	0	0
Asteraceae	0	1	0	0	1	0	1	5	0	1	4	0
Betula	0	0	0	1	0	0	1	0	1	0	2	1
Brassicaceae	0	0	0	1	0	0	0	0	0	0	0	0
Cannabaceae	0	0	0	0	0	0	0	0	0	0	0	0
Carpinus	0	0	0	0	0	0	0	0	0	0	0	0
Caryophyllaceae	0	0	0	0	0	0	0	0	0	0	0	0
Castanea	0	0	0	0	0	0	0	5	0	0	0	0
Centaurea	0	0	0	0	0	0	0	0	0	0	0	0
Corylus	0	0	0	0	0	0	0	0	0	0	0	0
Cupressaceae	1	0	0	0	1	0	2	0	1	0	2	1
Cyperaceae	0	0	0	0	0	0	0	0	0	0	0	0
Fabaceae	0	3	0	0	0	0	0	1	0	1	0	0
Fagus	0	0	0	0	0	0	0	1	0	0	0	0
Fraxinus	0	0	0	0	0	0	0	1	0	0	0	0
Ginkgo	0	0	0	0	0	0	0	0	0	0	0	0
Humulus	0	1	0	0	0	0	0	0	0	1	0	0
Impatiens	0	0	0	1	0	0	0	0	0	0	0	0
Indet	1	4	0	0	2	0	2	5	0	1	6	0
Juglans	0	0	0	0	0	0	0	0	0	0	0	0
Ligustrum	0	0	0	0	0	0	0	0	0	0	0	0
Mercurialis	1	5	0	1	1	0	15	4	0	1	5	0
Morus	0	0	0	0	0	0	0	0	0	0	0	0
Ostrya	0	0	0	0	0	0	0	0	0	0	0	0
Phacelia	0	1	0	0	3	0	1	0	0	0	0	0
Philadelphus	0	0	0	0	0	0	0	0	0	0	0	0
Picea	0	0	0	1	0	0	0	1	1	0	0	0
Pinus	1	1	0	0	0	0	1	1	0	0	2	0
Plantago	3	3	0	8	3	0	11	25	0	22	31	0
Platanus	0	0	0	0	0	0	0	0	0	0	0	0
Poaceae	4	9	2	6	15	0	9	42	2	12	69	2
Polygonaceae	0	4	0	0	2	0	0	0	0	0	39	0
Populus	0	0	0	0	0	0	0	0	0	0	0	0
Quercus	0	0	0	0	0	0	0	2	0	0	0	0
Ranunculaceae	0	0	0	0	0	0	0	0	0	0	0	0
Rosaceae	0	0	1	0	0	0	0	0	0	4	0	0
Rubiaceae	0	1	0	0	0	0	0	0	0	0	0	0
Rumex	0	4	0	0	1	0	1	1	0	2	4	0
Salix	0	0	0	0	0	0	0	0	0	0	0	0
Sambucus	0	0	0	0	0	0	0	0	0	0	0	0
Secale	0	0	0	0	0	0	0	0	0	0	0	0
Syringa	0	0	0	0	0	0	0	0	0	0	0	0
Tamarix	0	0	0	0	0	0	0	0	0	0	0	0
Tilia	0	0	0	0	0	0	1	0	1	1	0	0
Triticum	0	0	0	0	0	0	0	0	0	0	0	0
Typhaceae	0	0	0	0	0	0	0	0	0	0	0	0
Ulmus	0	0	0	0	0	0	0	0	0	0	0	0
Urticaceae	0	8	3	6	25	3	5	83	3	7	12	1
Xantium	0	0	0	0	0	0	0	1	0	0	0	0
Zea Mays	0	0	0	0	1	0	3	1	1	0	0	0

3.1.2.2 Detailed analyses of the early bloomer season

The early bloomer season, represented by the measurement in 2019, revealed following exposure levels on horse grounds (**Table 01**).

On the pasture of testing side **A** 69 grains per m³ *Alnus*-, 21 grains per m³ *Corylus*- and 13 grains per m³ *Cupressaceae* pollen were measured. Whereas on the paddock 133 grains per m³ *Alnus*-, 38 grains per m³ *Corylus*- and 21 grains per m³ *Cupressaceae* pollen were detected. *Juglans*- and *Plantago* pollen were only found at the pasture and *Fagus*-, *Picea*- and *Poaceae* pollen only on the paddock. **If the pollen were found at both testing locations the exposure levels on the paddock were higher in comparison with the pasture.**

On testing side **B**, on the pasture 169 grains per m³ *Cupressaceae*-, 121 grains per m³ *Alnus*- and 27 grains per m³ *Corylus* pollen were captured. Compared to the paddock, where 206 grains per m³ *Cupressaceae*-, 151 grains per m³ *Alnus*- and 45 grains per m³ *Corylus* pollen were trapped. Some pollen were only found on the paddock, *Aesculus*-, *Amaranthaceae*- and *Poaceae* pollen. **Regarding the exposure levels they were always higher on the paddock compared to the pasture.**

On the horse grounds of stable **C** the least amount of pollen were found in the early bloomer season. On the pasture 79 grains per m³ *Alnus*- and 20 grains per m³ *Corylus* pollen were captured. On the paddock 307 grains per m³ Alnus-, 66 grains per m³ *Corylus*- and 11 grains per m³ *Cupressaceae* pollen were trapped. **The exposure levels were higher on the paddock in comparison to the pasture.** *Picea*- and *Poaceae* pollen were only detected on the pasture, in contrast to *Acer*- and *Polygonaceae* pollen, which were only found on the paddock.

The measurement on study location **D** revealed, on the pasture 310 grains per m³ *Cupressaceae*-, 198 grains per m³ *Alnus*- and 41 grains per m³ *Corylus* pollen. On the paddock 499 grains per m³ *Cupressaceae*-, 293 grains per m³ *Alnus*-, 79 grains per m³ *Corylus*- and 17 grains per m³ *Populus* pollen were detected. **Compared to each other the pollen exposure levels are higher on the paddock, than on the pasture.** *Indet*, *Juglans* and *Tilia* pollen could only be found on the pasture, on the other side *Fabaceae*, *Fraxinus*, *Pinus* and *Salix* pollen only were captured on the paddock.

3.1.2.3 Detailed analyses of the middle bloomer season

During the middle bloomer season the greatest variety different pollen were detected (**Table 02**).

On the pasture of testing side **A**, 1 grain per m³ *Carpinus*-, *Picea*- and *Salix* pollen were captured, but on the paddock 13 grains per m³ *Quercus*-, 6 grains per m³ *Fraxinus*-, 5 grains per m³ *Betula*-, 3 grains per m³ *Brassicaceae*-, *Fagus*- and *Salix* pollen, 2 grains per m³ *Picea*- and *Platanus* pollen, 1 grain per m³ *Abies*-, *Plantago*- and *Rubiaceae* pollen were trapped. *Caprinus* pollen were only found at the pasture and *Abies*-, *Betula*-, *Brassicaceae*-, *Fagus*-, *Fraxinus*-, *Plantago*-, Platanus-, *Quercus*- and *Rubiaceae* pollen only on the paddock. **During that measurement the pollen exposure on the paddock also was higher than it was on the pasture.**

Testing side **B** had higher pollen exposure levels. On the pasture 90 grains per m³ *Quercus*-, 77 grains per m³ *Fagus*-, 59 grains per m³ *Picea*-, 51 grains per m³ *Betula*-, 17 grains per m³ *Fraxinus*-, 16 grains per m³ *Cupressaceae*-, 16 grains per m³ *Juglans*-, 14 grains per m³ *Salix*-, 13 grains per m³ *Brassicaceae*- and *Caryophyllaceae* pollen and 13 grains per m³ *Morus* pollen were measured. On the paddock 74 grains per m³ *Quercus*-, 44 grains per m³ *Fagus*-, 28 grains per m³ *Picea*-, 23 grains per m³ *Betula*-, 10 grains per m³ *Salix* pollen were detected. *Asteraceae*-, *Caryophyllaceae*-, *Corylus*-, *Indet*-, *Ostrya*-, *Ulmus*-, *Urticaceae* pollen could only be found at the pasture, but *Apiaceae* pollen only on the paddock. **At these measurement points most of the exposure levels are higher on the pasture, than on the paddock, except for *Platanus*-, *Populus*-, *Alnus*-, *Rosaceae* pollen.**

Stable **C** showed, on the pasture, 42 grains per m³ *Picea* pollen. In contrast to the paddock with 121 grains per m³ *Picea*-, 82 grains per m³ *Quercus*-, 37 grains per m³ *Betula*-, 33 grains per m³ *Fagus*-, 12 grains per m³ *Fraxinus*- and 11 grains per m³ *Juglans* pollen were measured. *Picea* pollen were the only ones detected on the pasture whereas *Quercus*-, *Betula*-, *Fagus*-, *Fraxinus*- and *Juglans* pollen only on the paddock. **Overall the exposure was higher on the paddock.**

On the pasture, of study side **D**, 95 grains per m³ *Picea*-, 37 grains per m³ *Fagus*-, 33 grains per m³ *Quercus*-, 23 grains per m³ *Morus*- and 16 grains per m³ *Betula* pollen were captured. On the paddock 228 grains per m³ *Picea*-, 121 grains per m³ *Quercus*-, 79 grains per m³ *Fagus*-,

55 grains per m³ *Morus*-, 49 grains per m³ *Betula*- and 30 grains per m³ *Juglans* pollen could be detected. Some pollen from *Alnus*, *Apiaceae*, *Corylus*, *Ginkgo*, *Indet*, *Poaceae*, *Populus*, *Rosaceae*, *Rumex* and *Tamarix* only were captured on the paddock, but *Acer*- and *Brassicaceae* pollen only could be located at the pasture. **Overall on the paddock a higher exposure level was determined, compared to the pasture except for *Abies* pollen.**

3.1.2.4 Detailed analyses of the summer bloomer season

In the summer bloomer season, also called the grass season, the highest amount of *Poaceae* pollen were found (**Table 03**).

During this measurement on the pasture of stable **A** 88 grains per m³ *Poaceae*-, 43 grains per m³ *Urticaceae*- and 23 grains per m³ *Plantago* pollen were found. On the paddock 283 grains per m³ *Urticaceae*-, 55 grains per m³ *Poaceae*- and 22 grains per m³ *Sambucus* pollen were measured. Several kinds of pollen, like *Ailanthus*-, *Cannabaceae*-, *Castanea*-, *Centaurea*-, *Cyperaceae*-, *Fagus*-, *Morus*-, *Platanus*- and *Tilia* pollen were only detected on the pasture, on the other hand *Apiaceae*-, *Asteraceae*-, *Betula*-, *Carpinus*-, *Rumex*-, *Salix*-, *Secale*- and *Triticum* pollen only on the paddock. **The measured levels were higher on the paddock than on the pasture, except for *Indet*-, *Plantago*- and *Poaceae* pollen.**

At testing side **B** 84 grains per m³ *Poaceae*-, 54 grains per m³ *Plantago*- and 10 grains per m³ *Urticaceae* pollen were detected, on the pasture. On the paddock 71 grains per m³ *Poaceae*-, 25 grains per m³ *Urticaceae*- and 12 grains per m³ *Ailanthus* pollen were measured. *Betula*, *Carpinus*-, *Cupressaceae*-, *Picea*-, *Quercus*-, *Secale*- and *Typhaceae* pollen were only present at the pasture, on the other hand *Castanea*-, *Phacelia*-, *Ranunculaceae*-, *Rubiaceae*- and *Rumex* pollen only on the paddock. ***Plantago*-, *Poaceae*-, *Sambucus*- and *Tilia* pollen were captured in higher amounts on the pasture, than on the paddock, the rest found on both testing sides were in higher levels at the paddock.**

At stable **C**, on the pasture 362 grains per m³ *Poaceae*- and 17 grains per m³ *Urticaceae* pollen were detected. On the paddock 113 grains per m³ *Poaceae*-, 33 grains per m³ *Urticaceae*- and 10 grains per m³ *Sambucus* pollen were trapped. Some like *Apiaceae*-, *Fraxinus*-, *Ligustrum*-,

Ranunculaceae-, *Salix* pollen were only found on the pasture, whereas *Amaranthaceae*-, *Cannabaceae*-, *Philadelphus*-, *Rubiaceae*-, *Rumex*-, *Tilia* pollen only found on the paddock. **Most of the pollen are in higher concentrations on the paddock, except *Mercurialis*-, *Pinus*-, *Plantago*-, *Poaceae*- and *Quercus* pollen.**

The measurement at study side **D**, representing the grass season showed, on the pasture 61 grains per m³ *Urticaceae*-, 23 grains per m³ *Poaceae*- and 12 grains per m³ *Plantago* pollen were trapped. On the paddock 82 grains per m³ *Poaceae*-, 28 grains per m³ *Urticaceae*-, 14 grains per m³ *Plantago*, 10 grains per m³ *Pinus* pollen were detected. **Except for *Urticaceae* pollen, which were found in higher amounts on the pasture, the pollen exposure levels on the paddock are higher compared to the pasture.**

3.1.2.5 Detailed analyses of the late bloomer season

The late bloomer season was the season with the lowest exposure levels of captured pollen (**Table 04**).

Testing location **A** had a very low pollen exposure. On the pasture 4 grains per m³ *Poaceae*-, 3 grains per m³ *Ambrosia*- and *Plantago* pollen-, 1 grain per m³ *Amaranthaceae*-, *Artemisia*-, *Cupressaceae*-, *Indet*-, *Mercurialis*- and *Pinus* pollen were captured. On the paddock 12 grains per m³ *Amaranthaceae*-, 9 grains per m³ *Poaceae*-, 8 grains per m³ *Urticaceae*-, 5 grains per m³ *Mercurialis*-, 4 grains per m³ *Ambrosia*-, *Indet*-, *Polygonaceae*- and *Rumex* pollen, 3 grains per m³ *Fabaceae*- and *Plantago* pollen, 1 grain per m³ *Artemisia*-, *Asteraceae*-, *Humulus* , *Phacelia*-, *Pinus*- and *Rubiaceae* pollen were trapped. *Cupressaceae* pollen only could be detected at the pasture, whereas *Asteraceae*-, *Fabaceae*-, *Humulus*-, *Phacelia*-, *Polygonaceae*-, *Rubiaceae*, *Rumex*- and *Urticaceae* pollen only on the paddock. **The comparison between the testing sides revealed, that the paddock has a higher exposure level than the pasture.**

At study side **B**, on the pasture 8 grains per m³ *Plantago*-, 7 grains per m³ *Amaranthaceae*-, 6 grains per m³ *Poaceae*- and *Urticaceae* pollen, 2 grains per m³ *Ambrosia*-, 1 grain per m³ *Artemisia*-, *Betula*-, *Brassicaceae*-, *Impatiens*-, *Mercurialis*- and *Picea* pollen were measured. On the paddock 25 grains per m³ *Urticaceae*-, 15 grains per m³ *Amaranthaceae*- and *Poaceae* pollen,

3 grains per m³ *Artemisia*-, *Phacelia*- and *Plantago* pollen, 2 grains per m³ *Ambrosia*-, *Indet*- and *Polygonaceae* pollen, 1 grain per m³ *Asteraceae*-, *Cupressaceae*-, *Mercurialis*-, *Rumex*- and *Zea Mays* pollen were detected. Several pollen were just found on one of the testing locations, *Betula*, *Brassicaceae*-, *Impatiens*-, *Picea* pollen were only captured on the pasture and *Cupressaceae*-, *Indet*-, *Phacelia*-, *Polygonaceae*-, *Rumex*- and *Zea Mays* pollen only on the paddock. **The pollen count was either equal or higher on the paddock, except of the *Plantago* pollen exposure level.**

Also testing location **C** the exposure level was low. On the pasture are 15 grains per m³ *Mercurialis*-, 11 grains per m³ *Plantago*-, 9 grains per m³ *Amaranthaceae*- and *Poaceae* pollen, 5 grains per m³ *Urticaceae*-, 3 grains per m³ *Zea Mays*-, 2 grains per m³ *Ambrosia*-, *Cupressaceae*- and *Indet* pollen, 1 grains per m³ *Asteraceae*-, *Betula*-, *Phacelia*-, *Pinus*-, *Rumex*- and *Tilia* pollen were found. On the paddock 81 grains per m³ *Urticaceae*-, 42 grains per m³ *Poaceae*-, 25 grains per m³ *Plantago*-, 10 grains per m³ *Amaranthaceae*-, 5 grains per m³ *Asteraceae*-, *Castanea*- and *Indet* pollen, 4 grains per m³ *Mercurialis*-, 2 grains per m³ *Ambrosia*- and *Quercus* pollen, 1 grain per m³ *Artemisia*-, *Fabaceae*-, *Fagus*-, *Fraxinus*-, *Picea*-, *Pinus*-, *Rumex*-, *Xantium*- and *Zea Mays* pollen were detected. *Betula*-, *Cupressaceae*-, *Phacelia*-, *Tilia* pollen were only measured on the pasture and *Artemisia*-, *Castanea*-, *Fabaceae*-, *Fagus*-, *Fraxinus*-, *Picea*-, *Quercus*- and *Xantium* pollen only on the paddock. **The pollen exposure was higher on the paddock, than it was on the pasture except for *Mercurialis*- and *Zea Mays* pollen.**

Stable **D** showed, on the pasture, 22 grains per m³ *Plantago*-, 12 grains per m³ *Poaceae*-, 8 grains per m³ *Amaranthaceae*-, 7 grains per m³ *Urticaceae*-, 4 grains per m³ *Rosaceae*-, 2 grains per m³ *Rumex*-, 1 grain per m³ *Asteraceae*-, *Fabaceae*-, *Humulus*-, *Indet*-, *Mercurialis*- and *Tilia* pollen were measured. On the paddock 69 grains per m³ *Poaceae*-, 39 grains per m³ *Polygonaceae*-, 31 grains per m³ *Plantago*-, 12 grains per m³ *Urticaceae*-, 6 grains per m³ *Amaranthaceae*- and *Indet* pollen, 5 grains per m³ *Mercurialis*-, 4 grains per m³ *Asteraceae*- and *Rumex* pollen, 2 grains per m³ *Ambrosia*-, *Betula*-, *Cupressaceae*- and *Pinus* pollen and 1 grain per m³ *Alnus* pollen. *Alnus*, *Ambrosia*, *Betula*, *Cupressaceae*, *Pinus* and *Polygonaceae* were only found on the paddock, whereas *Fabaceae*, *Humulus*, *Rosaceae* and *Tilia* were only found

on the pasture. **Except *Amaranthaceae* pollen all the others, found on both testing sides, were found equally distributed or in higher amounts on the paddock than on the pasture.**

3.1.2.6 Detailed analyses of location APIS Vienna – reference location

Vienna is the reference location, therefore the appropriate time slot, depending on the time the measurement was done, was chosen as reference. So, there are four different time slots, representing the timepoint for each testing side, for all seasons (**Table 05a – 05d**).

The early bloomers are represented in **Table 05a**. At 08:00 - 12:00 3 grains per m³ *Urticaceae*-, 2 grains per m³ *Poaceae*-, 1 grain per m³ *Amaranthaceae*-, *Ambrosia*- and *Rosaceae* pollen, at 10:00 – 14:00 973 grains per m³ *Cupressaceae*-, 115 grains per m³ *Alnus*-, 12 grains per m³ *Corylus* , 1 grain per m³ *Picea*-, *Populus*- and *Ulmus* pollen, at 12:00 – 16:00 743 grains per m³ *Cupressaceae* , 91 grains per m³ *Alnus*-, 19 grains per m³ *Corylus*-, 1 grain per m³ *Picea*- and *Ulmus* pollen, at 14:00 – 18:00 223 grains per m³ *Cupressaceae*-, 116 grains per m³ *Alnus*-, 28 grains per m³ *Corylus* , 1 grain per m³ *Populus*- and *Ulmus* pollen were counted.

Table 5b shows the middle bloomer season. At 08:00 – 12:00 33 grains per m³ *Quercus*-, 20 grains per m³ *Fraxinus*-, 19 grains per m³ *Morus*-, 10 grains per m³ *Juglans*-, 9 grains per m³ *Cupressaceae*-, 8 grains per m³ *Picea*-, 7 grains per m³ *Fagus*-, 6 grains per m³ *Betula*- and *Platanus* pollen, 5 grains per m³ *Pinus*-, 3 grains per m³ *Aesculus*-, *Salix*- and *Tamarix* pollen, 2 grains per m³ *Carpinus*- and *Rosaceae* pollen, 1 grain per m³ *Abies*-, *Poaceae*-, *Populus*-, *Ranunculaceae*-, *Rumex* pollen, at 10:00 – 14:00 60 grains per m³ *Quercus*-, 46 grains per m³ *Cupressaceae*-, 42 grains per m³ *Morus*-, 34 grains per m³ *Fagus*-, 26 grains per m³ *Picea*-, 16 grains per m³ *Betula*- and *Juglans* pollen, 14 grains per m³ *Fraxinus*-, 13 grains per m³ *Pinus*-, 7 grains per m³ *Aesculus*-, 6 grains per m³ *Platanus*-, 4 grains per m³ *Carpinus*-, 3 grains per m³ *Salix*-, 2 grains per m³ *Ranunculaceae*-, *Rosaceae* and *Tamarix* pollen, 1 grain per m³ *Abies*-, *Alnus*-, *Ostrya*-, *Poaceae*-, *Populus*- and *Rumex* pollen, at 12:00 – 16:00 49 grains per m³ *Picea*-, 48 grains per m³ Cupressaceae-, 46 grains per m³ *Quercus*-, 45 grains per m³ *Morus*-, 40 grains per m³ *Fagus*-, 20 grains per m³ *Betula*-, 10 grains per m³ *Juglans*- and *Pinus* pollen, 7 grains per m³ *Aesculus*-, 6 grains per m³ *Fraxinus*- and *Platanus* pollen, 4 grains per m³

Carpinus- and *Rosaceae* pollen, 3 grains per m³ *Salix*- , 2 grains per m³ *Ranunculaceae*- and *Tamarix* pollen, 1 grain per m³ *Alnus*-, *Corylus*-, *Cyperaceae*-, *Ostrya*- and *Populus* pollen, at 14:00 – 18:00 53 grains per m³ *Picea*-, 30 grains per m³ *Morus*-, 30 grains per m³ *Quercus*-, 19 grains per m³ *Fagus*-, 16 grains per m³ *Betula*-, 12 grains per m³ *Cupressaceae*-, 7 grains per m³ *Juglans*-, 5 grains per m³ *Aesculus*- and *Pinus* pollen, 4 grains per m³ *Platanus*-, 3 grains per m³ *Fraxinus*-, *Rosaceae*- and *Salix* pollen, 2 grains per m³ *Tamarix*-, 1 grain per m³ *Alnus*-, *Carpinus*-, *Corylus*-, *Cyperaceae*-, *Ostrya*-, *Plantago*-, *Poaceae*- and *Rumex* pollen were detected.

During the grass season (**Table 05c**) following pollen were trapped. At 08:00 – 12:00 21 grains per m³ *Poaceae*-, 7 grains per m³ *Tilia*-, 4 grains per m³ *Plantago*-, 3 grains per m³ *Urticaceae*-, 2 grains per m³ *Pinus*-, 1 grain per m³ *Ailanthus*-, *Cupressaceae*-, *Picea*-, *Rosaceae*-, *Rubiaceae*-, *Salix*-, *Sambucus*- and *Typhaceae* pollen, at 10:00 – 14:00 26 grains per m³ *Poaceae*- , 12 grains per m³ *Tilia*-, 4 grains per m³ *Plantago*- and *Urticaceae* pollen, 2 grains per m³ *Ailanthus*-, *Picea*- and *Sambucus* pollen, 1 grain per m³ *Pinus*-, *Rosaceae*-, *Rubiaceae*- and *Salix* pollen, at 12:00 – 16:00 14 grains per m³ *Poaceae*-, 9 grains per m³ *Tilia*-, 4 grains per m³ *Urticaceae*-, 3 grains per m³ *Plantago*-, 2 grains per m³ *Ailanthus*-, 1 grain per m³ *Carpinus*-, *Castanea*-, *Quercus*- and *Rubiaceae* pollen, at 14:00 – 18:00 10 grains per m³ *Poaceae*-, 8 grains per m³ *Urticaceae*-, 2 grains per m³ *Ailanthus*- and *Tilia* pollen, 1 grain per m³ *Castanea* , *Philadelphus*-, *Pinus*-, *Plantago*- and *Quercus* pollen were detected.

The results of the late bloomers season are graphically presented in **Table 05d**. At 08:00 – 12:00 3 grains per m³ *Urticaceae*-, 2 grains per m³ *Poaceae*-, 1 grain per m³ *Amaranthaceae*-, *Ambrosia*- and *Rosaceae* pollen, at 10:00 – 14:00 3 grains per m³ *Urticaceae*-, 1 grains per m³ *Amaranthaceae* pollen, at 12:00 – 16:00 3 grains per m³ *Urticaceae*-, 2 grains per m³ *Poaceae*- 1 grain per m³ *Betula* , *Cupressaceae*-, *Picea*-, *Tilia*- and *Zea Mays* pollen, at 14:00 – 18:00 2 grains per m³ *Poaceae*-, 1 grain per m³ *Betula*-, *Cupressaceae*- and Urticaceae pollen were captured.

Table 05a: List of captured pollen in Vienna during the early bloomer measurement. The four time slots were used as reference for each testing side at the same time.

low counts < 10 grains / m³, middle counts 10 – 100 grains / m³ high counts < 100 grains / m³

early bloomers	0800-1200	1000-1400	1200-1600	1400-1800
Alnus	0	115	91	116
Amaranthaceae	1	0	0	0
Ambrosia	1	0	0	0
Corylus	0	12	19	28
Cupressaceae	0	973	743	223
Picea	0	1	1	0
Poaceae	2	0	0	0
Populus	0	1	0	1
Rosaceae	1	0	0	0
Ulmus	0	1	1	1
Urticaceae	3	0	0	0

Table 05b: List of captured pollen in Vienna during the middle bloomer measurement. The four time slots were used as reference for each testing side at the same time.

low counts < 10 grains / m³, middle counts 10 – 100 grains / m³, high counts < 100 grains / m³

middle bloomers	0800-1200	1000-1400	1200-1600	1400-1800
Abies	1	1	0	0
Aesculus	3	7	7	5
Alnus	0	1	1	1
Betula	6	16	20	16
Carpinus	2	4	4	1
Corylus	0	0	1	1
Cupressaceae	9	46	48	12
Cyperaceae	0	0	1	1
Fagus	7	34	40	19
Fraxinus	20	14	6	3
Juglans	10	16	10	7
Morus	19	42	45	30
Ostrya	0	1	1	1
Picea	8	26	49	53
Pinus	5	13	10	5
Plantago	0	0	0	1
Platanus	6	6	6	4
Poaceae	1	1	0	1
Populus	1	1	1	0
Quercus	33	60	46	30
Ranunculaceae	1	2	2	0
Rosaceae	2	2	4	3
Rumex	1	1	0	1
Salix	3	3	3	3
Tamarix	3	2	2	2

Table 05c: List of captured pollen in Vienna during the summer bloomer measurement. The four time slots were used as reference for each testing side at the same time.

low counts < 10 grains / m³, middle counts 10 – 100 grains / m³, high counts < 100 grains / m³

summer bloomers	0800-1200	1000-1400	1200-1600	1400-1800
Ailanthus	1	2	2	2
Carpinus	0	0	1	0
Castanea	0	0	1	1
Cupressaceae	1	0	0	0
Philadelphus	0	0	0	1
Picea	1	2	0	0
Pinus	2	1	0	1
Plantago	4	4	3	1
Poaceae	21	26	14	10
Quercus	0	0	1	1
Rosaceae	1	1	0	0
Rubiaceae	1	1	1	0
Salix	1	1	0	0
Sambucus	1	2	0	0
Tilia	7	12	9	2
Typhaceae	1	0	0	0
Urticaceae	3	4	4	8

Table 05d: List of captured pollen in Vienna during the late bloomer measurement. The four time slots were used as reference for each testing side at the same time.

low counts < 10 grains / m³, middle counts 10 – 100 grains / m³, high counts < 100 grains / m³

late bloomers	0800-1200	1000-1400	1200-1600	1400-1800
Amaranthaceae	1	2	0	0
Ambrosia	1	0	0	0
Betula	0	0	1	1
Cupressaceae	0	0	1	1
Picea	0	0	1	0
Poaceae	2	0	2	2
Rosaceae	1	0	0	0
Tilia	0	0	1	0
Urticaceae	3	3	3	1
Zea Mays	0	0	1	0

3.2 Qualitative analyses of captured pollen

3.2.1. Protein concentration of the extracts

The first challenge was to extract the captured material out of the filters. Thereafter, the protein concentration in the extracts were measured. The average protein concentration was 0.4087 µg / µl with a standard deviation of 0.227 µg / µl. The lowest protein concentration was 0.19 µg / µl in an extract captured during the early blooming season at location A. Whereas the highest concentration was captured in the late blooming season at location D, with 1.26 µg / µl (**Table 06**). Due to the differences, which were found throughout the extracts, the protein was adjusted during the SDS PAGE.

Table 06: Protein concentration of each sample determined by the NanoPhotometer® P 300.
E… early bloomers, M… middle bloomers, G… grass season, L… late bloomers; 1… paddock, 2… pasture

Study side	Sample ID	OD 260	OD 280	OD 320	mg/ml µg/µl	µg/10µl	total extract [µl]	total protein [µg]
A	E1	0.043	0.03	0.011	0.19	1.9	250	47.50
A	E2	0.05	0.033	0.012	0.21	2.1	240	50.40
A	M1	0.116	0.079	0.03	0.49	4.9	230	112.7
A	M2	0.074	0.062	0.032	0.3	3.0	550	165.0
A	G1	0.083	0.057	0.017	0.24	2.4	200	48.00
A	G2	0.057	0.041	0.017	0.24	2.4	750	180.0
A	L1	0.186	0.126	0.047	0.79	7.9	070	55.30
A	L2	0.051	0.038	0.017	0.21	2.1	340	71.40
B	E1	0.154	0.124	0.064	0.6	6.0	240	144.0
B	E2	0.095	0.068	0.029	0.39	3.9	230	89.70
B	M1	0.06	0.052	0.017	0.35	3.5	1500	525.0
B	M2	0.057	0.045	0.015	0.3	3.0	900	270.0
B	G1	0.118	0.091	0.036	0.55	5.5	250	137.5
B	G2	0.146	0.114	0.046	0.68	6.8	250	170.0
B	L1	0.067	0.043	0.016	0.27	2.7	250	67.50
B	L2	0.068	0.052	0.021	0.31	3.1	250	77.50

Continuation Table 06: Protein concentration of each sample determined by the NanoPhotometer® P 300.
E… early bloomers, M… middle bloomers, G… grass season, L… late bloomers; 1… paddock, 2… pasture

C	E1	0.053	0.007	0.014	0.23	2.3	250	57.50
C	E2	0.076	0.054	0.024	0.3	3.0	230	69.00
C	M1	0.045	0.033	0.012	0.21	2.1	750	157.50
C	M2	0.086	0.062	0.023	0.39	3.9	380	148.20
C	G1	0.161	0.123	0.061	0.62	6.2	140	86.80
C	G2	0.077	0.059	0.025	0.34	3.4	750	255.0
C	L1	0.062	0.047	0.018	0.29	2.9	250	72.50
C	L2	0.068	0.044	0.016	0.28	2.8	250	70.00
D	E1	0.145	0.096	0.036	0.6	6.0	070	42.00
D	E2	0.177	0.117	0.046	0.71	7.1	070	49.70
D	M1	0.069	0.046	0.017	0.29	2.9	350	101.5
D	M2	0.049	0.033	0.01	0.23	2.3	250	57.50
D	G1	0.091	0.06	0.023	0.37	3.7	100	37.00
D	G2	0.057	0.041	0.018	0.23	2.3	500	115.0
D	L1	0.096	0.085	0.024	0.61	6.1	250	152.5
D	L2	0.247	0.16	0.034	1.26	12.6	140	176.4

3.2.2. Molecular masses of the captured proteins

To analyse the pollen qualitatively, an SDS PAGE as well as a Western blot was performed. Silver staining was used to make the protein patterns visible, as Coomassie blue was not sensitive enough. SDS PAGE-separated proteins revealed different patterns for each season, location A (**Figure 10**) location B (**Figure 11**), location C (**Figure 12**) and location D (**Figure 13**).

The strongest protein patterns were visible during the late- and the early bloomer measurement, especially at location C and D. In detail, proteins with around 72 kilo Dalton (kDa) could be detected at location A in the middle- (M1) and the late bloomer season (L1) on the paddock, at location C during the grass season on the pasture (G2), at location D it was measured in the late bloomer season on both testing sides (L1, L2) and in the early bloomer season on the pasture (E2). On the paddock of location A and B, in the middle bloomer season (M1) proteins with around 55

kDa were visible. Proteins with around 34 kDa could be found at the paddock of stable A in the late bloomer season (L1) and stable C during the early bloomer measurement (E1). Further at stable B in the middle- and the late bloomer season on both testing sides (M1, M2, L1, L2) proteins with around 30 kDa were found. During the late bloomers at location A on the paddock (L1) and at location D on the pasture in the late- and early bloomer measurement (L2, E2) a protein with around 26 kDa was visible. The major focus was on Bet v 1, therefore protein with around 17 kDa were important and could indeed be detected at stable A and B on the paddock during the late bloomer season (L1) and at stable C on the pasture (L2), also during the early bloomer season it was found at location B on both sides (E1, E2) and C on the paddock (E1), at stable B it was found also in the grass season on the pasture (G2). The measuring point with the highest amount of different proteins was the paddock of location D in the late bloomer season (L1). Bands of larger proteins with around 95 kDa, 80 kDa and 72 kDa could be found, as well as proteins with around 60 kDa, 55kDa and 50 kDa.

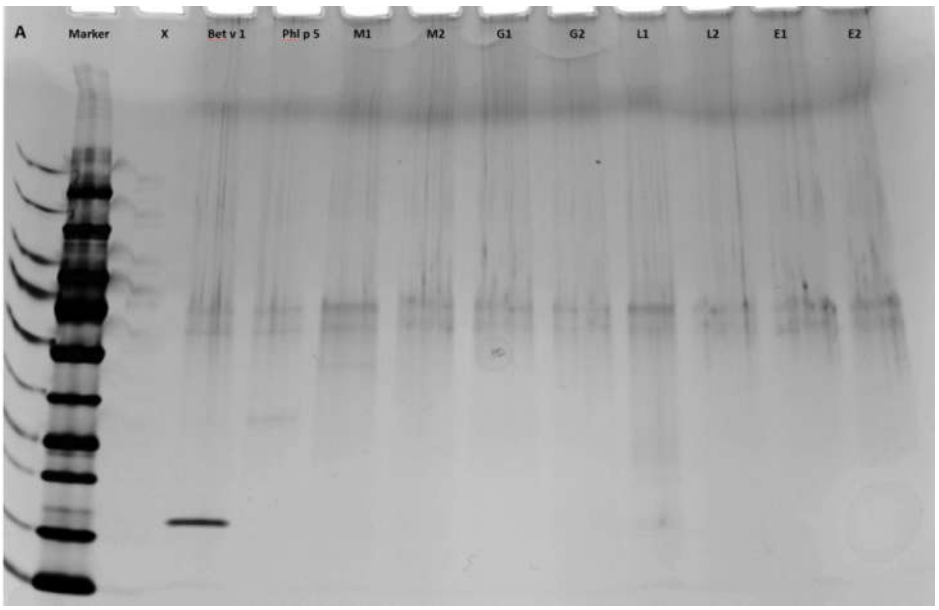

Figure 10: Protein patterns of location A at all seasons. Bet v 1…positive control, Phl p 5… positive control, M…middle bloomers, G…grass season, L…late bloomers, E…early bloomers; 1…paddock, 2…pasture

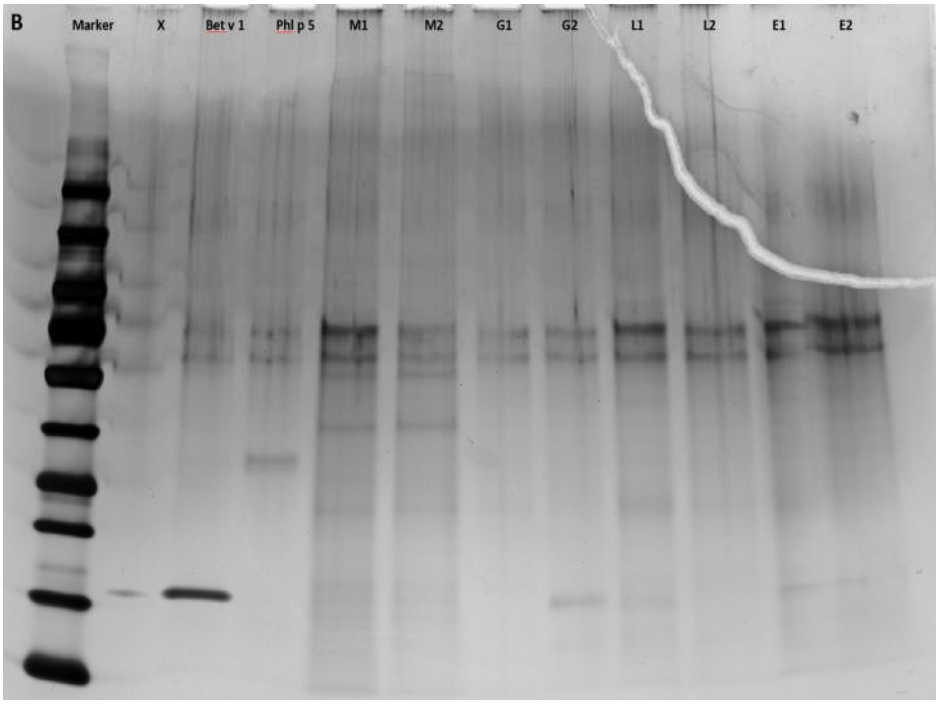

Figure 11: Protein patterns of location B at all seasons. Bet v 1…positive control, Phl p 5… positive control, M…middle bloomers, G…grass season, L…late bloomers, E…early bloomers; 1…paddock, 2…pasture

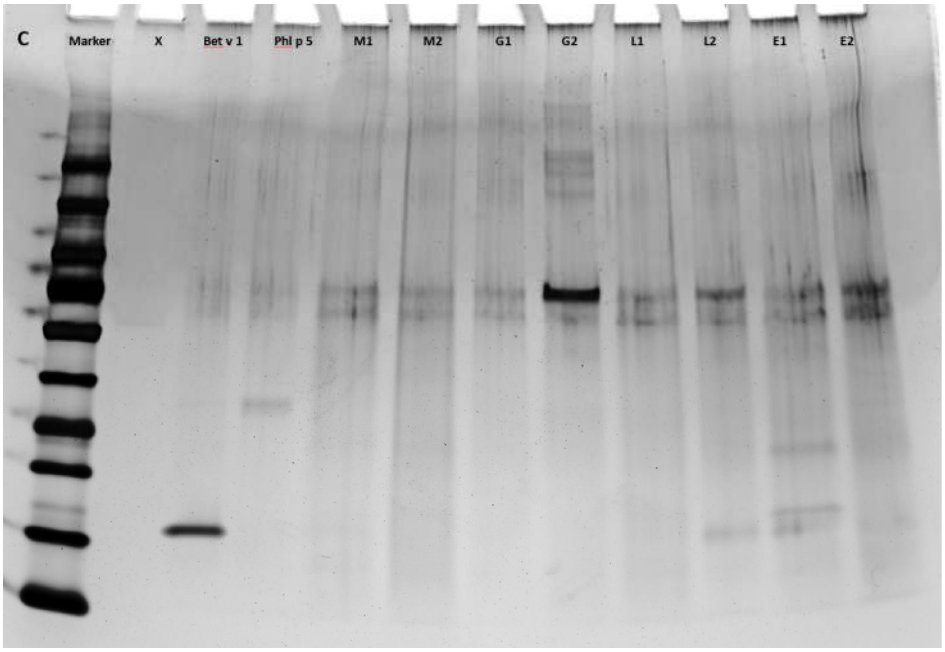

Figure 12: Protein patterns of location C at all seasons. Bet v 1…positive control, Phl p 5… positive control, M…middle bloomers, G…grass season, L…late bloomers, E…early bloomers; 1…paddock, 2…pasture

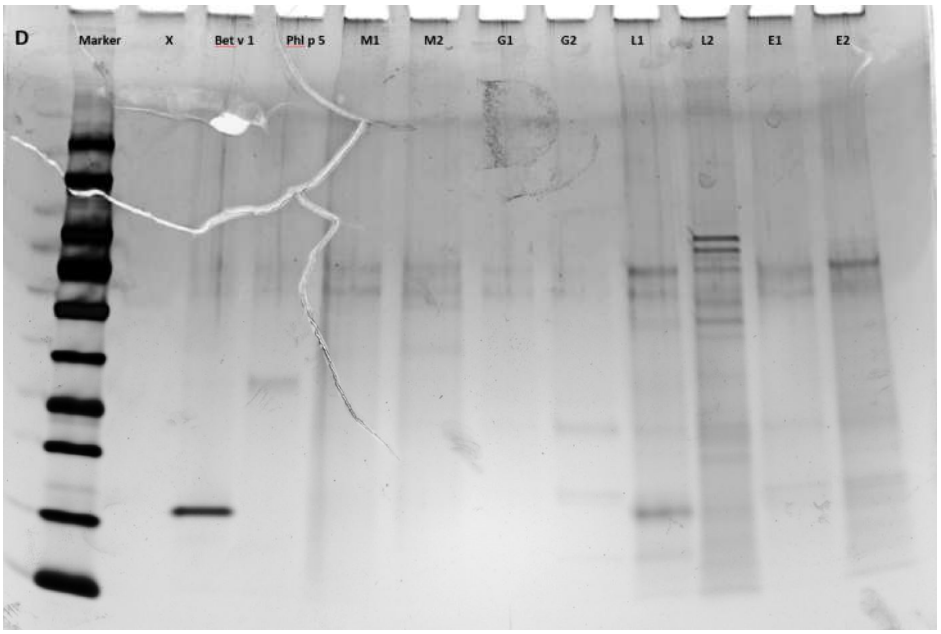

Figure 13: Protein patterns of location D at all seasons. Bet v 1…positive control, Phl p 5… positive control, M…middle bloomers, G…grass season, L…late bloomers, E…early bloomers; 1…paddock, 2…pasture

3.2.3. Bet v 1 antigen detection

The Western blot was performed to verify if the proteins, detected by the SDS PAGE, with the molecular weight around 17 kDa (Bet v 1 monomer), are Bet v 1 antigens.

The Western blot could not detect Bet v 1 in any of the samples, probably due to limits in sensitivity, just the positive control indicated that the blot was working properly. The picture is composed, the ethidium bromide mode and chemiluminescence mode were merged, to visualise the marker and the samples (**Figure 14**).

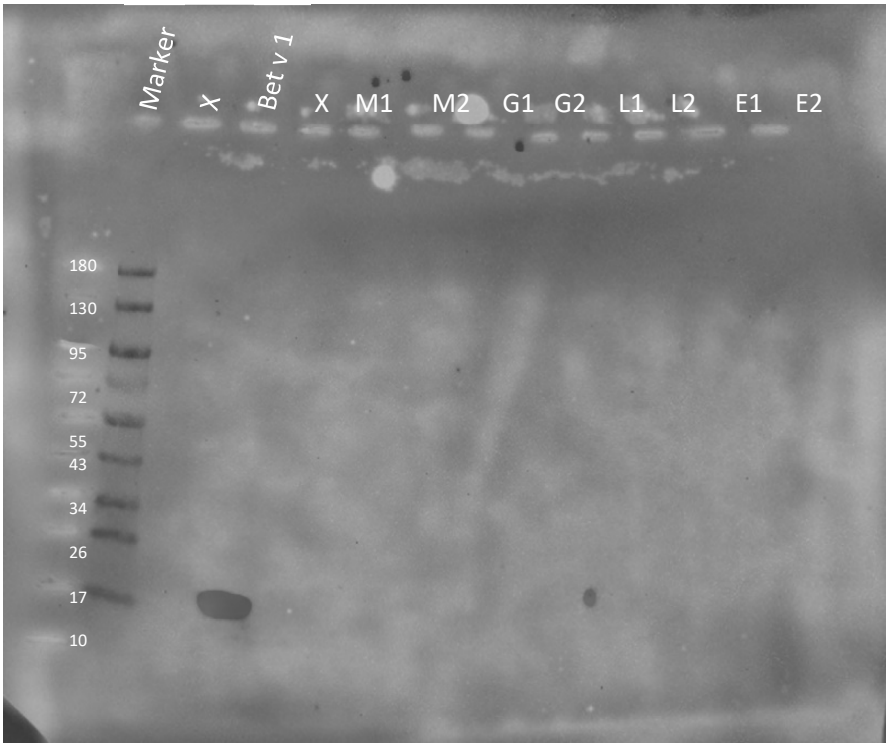

Figure 14: Western blot of all seasons at location C.

Bet v 1…positive control,
M …middle bloomers,
G…grass season,
L…late bloomers,
E…early bloomers;
1…paddock, 2…pasture

4. DISCUSSION

A previous study from our group using an allergen microarray revealed that the IgE antibody profiles between horses and humans significantly differ from each other [1]. For instance, while humans show IgE to birch pollen major allergen Bet v 1, the alder pollen allergen Aln g 1 seemed to be a much more relevant frequent sensitizer for horses.

The fact that sensitization is directly correlated with specific exposure in a certain environment prompted the research question of this master thesis: It was aimed to, for the first time, to compare the human urban pollen exposure with a typically equine environment, paddocks and pastures, of four paradigmatic horse riding stables on the countryside outside Vienna. Our collaborators from the Austrian Pollen Information Service were excellent partners in the field studies, contributing their profound aerobiology and botanical expertise to the study, besides the reference pollen counts from the city of Vienna as an important reference resource.

A flowering season is defined by a pollen exposure load of 100 grains per m³ or more, which could not be reached in all measurements in the different areas of the horse grounds [22]. The lead pollen of each season was found at all locations including the reference location Vienna, but the number of species and amounts of captured pollen differed substantially between the four testing sites around Vienna. Overall it could be verified, that the main pollen of each season were present at all testing locations. *Alnus*, *Corylus* and *Cupressaceae* pollen were representative for the early bloomer measurement. The middle bloomer season *Betula*, *Fagus*, *Fraxinus*, *Picea* and *Quercus* were identified as the major pollinators. Of course, *Poaceae* and *Urticaceae* were the most important plant in the grass season. *Amaranthaceae*- and *Plantago* pollen represent the late bloomer measurement, also *Poaceae* and *Urticaceae* pollen were detected, but in lower levels compared to the measurement in June.

The quantitative highly different pollen occurrence between the tested stables, can be explained by the fact that pollen exposure levels are highly depending on the individual plant species residing in the close vicinity. Whenever plants were found flowering or faded in the surroundings, at least

small amounts of their pollen were detected in the quantitative measurement, except for the middle bloomer season. *Poaceae* at location A and *Ranunculaceae* at stable D were botanically characterized, but none of their pollen were present in the trap.

To enhance comparability, we took the mean of all pastures and paddocks to compare it with the mean of all reference measurements at Vienna.

During the early bloomer measurement, the threshold of 100 grains per m³ was fulfilled at least once at each stable (**Table 01**).

In this season the major pollen found were *Alnus*, *Corylus* and *Cupressaceae*. The pollen exposure levels of *Alnus* pollen were highest at the paddocks compared to the pastures and Vienna. As previously shown Aln g 1 from alder is one of the most important allergens to horses, which could be explained by the overall higher exposure on horse grounds compared to the urban environment. The highest exposure of *Cupressaceae* was measured at Vienna, it also could be detected in high amount at the paddocks compared to the pastures.

Whereas during the middle bloomer measurement, the threshold of 100 grains per m³ was reached just at two of the testing locations stable C and D (**Table 02**).

The major pollen counts were derived from *Picea*, *Quercus*, *Fagus* and Betula. *Picea* was detected with the highest exposure during this flowering season. Overall the exposure on the paddocks were mostly the highest. *Betula* is the major allergen for humans, but for horses it seems less relevant. This could be explained by the fact that the *Betula* pollen exposure levels were lower on the tested horse grounds, even if they were found flowering nearby (stable A and stable B), compared to the *Alnus* exposure in the early bloomer season for example. It also must be taken into account that in 2018, the peak of the *Betula* pollen load was in the middle of April and our measurement took place at the end of April, so the pollen load was not that high anymore in general [23].

In the summer bloomer season the 100 grains per m³ was reached at two of the testing locations stable A and C (**Table 03**).

During the measurements predominantly the major allergenic *Poaceae* pollen were detected on the pastures, as expected. In contrast, *Urticaceae* pollen were captured in higher amounts on the paddocks. Except for stable D, where exceptionally *Poaceae* pollen were found in higher amounts on the paddock and *Urticaceae* pollen more on the pasture. The simple explanation may be that the grass on this pasture had been mowed before the measurement took place.

In terms of allergenicity in a previous study we reported that Phl p 5 of *Phleum pratense* is the major allergen to humans and Cyn d 1 from *Cynodon dactylon* to horses [1]. Due to the methodological limitation of our study could not differentiate between the two grass species, preventing any conclusions whether *Poaceae* pollen are really dominant on horse grounds compared to the human urban environment in Austria. In 2018 the peak of the flowering season of *Pocaceae* was at the end of May, which is a bit earlier than expected our measurement was at the 1st of June so we were shortly after the highest pollen load, but their pollen still were present [23].

During the late bloomer season the 100 grains per m³ was not reached at any measuring point (**Table 04**).

An explanation for the overall low pollen counts in the late bloomer season could be that plants like *Artemisia* and *Ambrosia* had their flowerings peak early in 2018, so in September, when our measurement took place the exposure levels were lower than usually [23].

The pollen exposure at the testing locations A and also B was low compared to the other stables, due to the fact that the measuring time was early in the morning and the pollen exposure varies over the day [24, 25]. In addition the results from the testing location Vienna, where the threshold of 100 grains per m³ was reached during the early bloomer measurement at 10:00 – 14:00 where 973 grains per m³ *Cupressaceae*- and 115 grains per m³ *Alnus* pollen were measured, at 12:00 – 16:00 with 743 grains per m³ *Cupressaceae* pollen and 14:00-18:00 with 223 grains per m³ *Cupressaceae*-, 116 grains per m³ *Alnus*- pollen, confirmed that there are diurnal differences in the pollen exposure levels throughout the day. *Ambrosia* even has elevated pollination during the night-time [25-27]. Studies also state, that the diurnal top levels of exposure are associated to a high

temperature and low humidity [24]. The urban human environment temperatures are higher than at the rural area, therefore prompting the distribution of pollen in cities.

The measurements were performed close to the ground, at the height of 28 cm. Except for the grass season the pollen exposure levels on the paddocks were mostly higher compared to the exposure levels on the pastures. Paddocks have plain grounds without vegetation allowing travelling with the wind, while pollen might be absorbed in the vegetation of pastures instead. This may mirror the higher pollen exposure levels in a city like Vienna: because of the sealed grounds in the urban deserts pollen are not stopped and can travel with the wind. Indeed, except the early bloomer measurement, the urban exposure levels were lower compared to the exposure levels at the horse grounds. Moreover, at the countryside, in typical equine environments, generally a greater variety of pollen was present and the exposure levels differed compared to the urban environment.

The study has some limitations. Only two portable pollen traps were available and a generator was needed to provide power on two of the pastures. Therefore, it was not possible to measure at more than one location with both testing sides at the same time, resulting in measurements at different daytimes among the stables. The comparability could have been improved if parallel measurements had been possible. On the one hand the diurnal fluctuations during the measurement and on the other hand slight changes in weather would not influence the results, because the reference location measured throughout the whole day and the fitting daytime was used for comparison. Also, accidental mowing at location D just before our measurements altered the results of the measurements.

For the qualitative measurements method for collection of indoor dust samples was adapted to outdoor measurements. Dustream® filters are usually used to capture indoor allergen such as by vacuuming matrasses, in this study we vacuumed the air by pointing the attached filter against the wind to capture flying aeroallergens. A mixture of pollen and other constituents in the air, such as insects, contributed to the total protein load on the filters. Indeed, differences were found in the total protein concentrations between the samples, which did not correlate with the quantitative pollen levels measured by the APIS. The lowest amount of protein was found in the early bloomer

season at location A, quantitatively the early bloomer season was the one with the highest pollen counts. The highest protein concentration was in the extract from the late bloomer measurement at location D, the quantitative pollen exposure was the lowest in this season. Due to the sampling method also some samples contained only low protein amounts.

To extract the proteins from collected samples a well-established protocol was used with following modifications [21, 28]. To protect the proteins during the lyophilization trehalose was used, instead of BSA, because it would have interfered with the further experiments.

SDS PAGE was performed to separate the sampled proteins according to their molecular mass. Separated proteins were visualized by silver staining instead of Coomassie stain due to low protein concentration. Different protein patterns were detected among the samples, also showing seasonal differences, in accordance with the seasonally higher pollen protein load in the air. Some samples L1 of stable A and B, L2 of stable C, E1 and E2 of stable B, E1 of stable C and G2 of stable B showed protein with around 17 kDa which corresponds with the mass of the major birch pollen allergen Bet v 1 as monomer. To verify whether these proteins were indeed Bet v 1 a Western blot was performed using a specific anti-Bet v 1 antibody. While Bet v 1 was detected in the positive control, the amount of Bet v 1 in the samples was too low for detection. Vacuuming was limited to 3 min at each testing site in this study. It was not possible to increase the sampling times due to a technical hurdle, overheating of the vacuum cleaner.

To conclude, this study showed that the pollen exposure correlates strongly with the local flora. It also showed that the lead pollen in each season were present at all locations. Overall the pollen load was surprisingly higher on the paddock compared to the pastures, and it was lower in the morning measurement compared to other performed during the rest of the day.

The “Pollen App” created by the Austrian Pollen Information Service, uses several pollen measuring locations in urban environments all over Austria, and the major pollen were present at all locations. Consequently, we conclude that if the owners know the specific allergens relevant for their horses the “human” Pollen-App may be a useful tool. To diagnose pollen allergies in horses, like in human allergic patients the clinical signs and history must be correlated with a type I skin

test or positive reactivity in a serum IgE test [29, 30]. Novel tests like the ISAC131 microarray may be effective screening approaches and need only low amounts of blood [1]. Upon specific diagnosis, and the horse owner has two options, i) allergen avoidance if possible, or ii) specific allergen immunotherapy which is the only causative treatment, but very cost-intensive [31].

The results of this master thesis strongly suggest that the Pollen-App can be used by owners of allergic horses to predict the upcoming pollen load at the stable and help with the avoidance. For instance, during day times of high load of the specific pollen, the owner should decide not to do heavy exercising with the horse as usually physical exercise associated with heavy breathing may induce more severe symptoms. Also, the daytime spent on the paddock and pastures could be adapted to lower pollen exposure, for example as our data show that the pollen load usually is higher on the paddock, the horse could spend more time on the pasture, or indoors. In contrast, if you have a horse which reacts to grass pollen, during summer bloomer season the horse should spend more time on the paddock to avoid spending long times on flowering pastures.

5. SUMMARY

5.1. English

We have previously demonstrated that horses exhibit other IgE sensitization profiles to allergens than humans. When 56 horse sera were tested in ISAC131 allergen microarray test, IgE antibodies were mostly directed against buckwheat allergen Fag e 2, and pollen allergens Aln g 1 from alder and Cyn d 1 from Bermuda grass [1].

We hypothesize in this study that the distinct sensitization profiles may be due to differences in allergen exposure. We established a collaboration with the Austrian pollen information service (APIS) to exactly measure pollen on paddocks and pastures. Therefore, the aims of the study are to identify pollen exposure on horse grounds, correlate allergen exposure with local plants, immunologically analyse captured allergens, compare pollen relevant for horses or humans and reveal if pollen App is suitable for horses.

For the study four horse stables in Lower Austria were selected for measurements over flowering seasons of a year to catch the early-, mid-, grass-, and late bloomers. For the pollen capturing a Burkard Hirst-type volumetric spore trap was placed 28 cm over the ground in a paddock or pasture, for one hour to collect the pollen of each season. Pollen were then counted and analysed palynologically. For the immunological characterization pollen were captured using a DUSTREAM® collector, attached to a Dyson® vacuum cleaner.

The pollen exposure on the horse grounds differ from the human urban environment and between the stables differences were detected, because the exposure levels are strongly linked with the local flora. The major pollen of each season could be captured at all testing sides. Overall the exposure at the horse grounds was higher, compared to the urban human environment. It was also higher at the paddocks compared to the pastures, except for the grass season, where the *Poaceae* pollen load was the highest at the pastures. The immunological analyses demonstrated seasonally different protein patterns. We conclude that the “Pollen – App” can be used by horse owner to predict the pollen load and help with the allergen avoidance.

5.2. German

Wir hatten in einer vorhergehenden Arbeit gezeigt, dass Pferde andere Sensibilisierungsprofile für Allergene aufweisen als Menschen. Es wurden 56 Pferdeseren mittels ISAC131-Allergen-Microarray-Test getestet, dabei wurden hauptsächlich IgE-Antikörper gegen das Buchweizenallergen Fag e 2 und die Pollenallergene Aln g 1 von Erle und Cyn d 1 von Bermuda-Gras gefunden [1]. In der vorliegenden Studie wurde die Hypothese aufgestellt, dass die unterschiedlichen Sensibilisierungsprofile auf Unterschiede in der Allergenexposition zurückzuführen sein könnten. In Zusammenarbeit mit dem Österreichischen Polleninformationsdienst haben wir vergleichend Pollen auf Koppeln und Weiden gemessen. Ziel der Studie ist es daher, die Pollenexposition in Pferdeställen zu identifizieren, die Allergenexposition mit der lokalen Flora zu korrelieren, eingefangene Allergene immunologisch zu analysieren, für Pferde oder Menschen relevante Pollen zu vergleichen und festzustellen, ob die Pollen App für Pferde und deren Besitzer geeignet ist.

Es wurden vier Pferdeställe in Niederösterreich für Messungen über ein Jahr ausgewählt, um Pollen der Früh-, Mittel-, Gras- und Spätblüher zu messen. Die quantitative Messung der Pollen wurde mittels volumetrischer Sporenfalle vom Typ Burkard Hirst durchgeführt, die eine Stunde lang auf einer 28 cm über dem Boden platziert war, um Pollen zu sammeln. Die Pollen wurden dann gezählt und palynologisch analysiert. Zur immunologischen Charakterisierung wurden Pollen mit einem DUSTREAM®-Kollektor, an einen Dyson® Staubsauger angeschlossen, aufgefangen.

Sowohl unterscheidet sich die Pollenexposition auf den Pferdehöfen von der städtischen Umgebung, aber auch zwischen den Ställen wurden Unterschiede festgestellt, da die Expositionsniveaus stark mit der lokalen Flora verknüpft sind. Jedoch die Hauptpollen jeder Saison konnte überall gefangen werden. Insgesamt war die Exposition auf dem Pferdehof im Vergleich zur städtischen menschlichen Umgebung höher. Im Vergleich zu den Weiden war sie auf den Koppeln höher, mit Ausnahme der Grassaison, in der die Pollenbelastung der *Poaceae* auf den Weiden am höchsten war. Die immunologischen Analysen zeigten saisonal unterschiedliche Proteinmuster. Wir konkludieren, dass die „Pollen App“ vom Pferdebesitzer genutzt werden kann, um die Pollenbelastung vorherzusagen und bei der Vermeidung zu helfen.

6. CURRICULUM VITAE

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Education:

2017 – 2019	Interdisciplinary Master in Human – Animal – Interactions at the Interuniversity Messerli Research Institute of the University of Veterinary Medicine Vienna
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2007 – 2011	Studium der Veterinärmedizin an der Veterinärmedizinischen Universität Wien – nicht abgeschlossen
1999 – 2007	Bundesgymnasium und Bundesrealgymnasium Wien VIII
1995 – 1999	Evangelische Volksschule Wien – Gumpendorf

Courses

2018	Ausbildung zur Ernährungsberaterin für Hunde

Conference presentations

November 8, 2019 Poster presentation “Pollen exposure levels on horse grounds and the urban human environment. A collaborative study with the Austrian Pollen Information Service.” Meeting of the Messerli Foundation Board at the interuniversity Messerli Research Institute, Vienna, Austria

May 22, 2019 Poster presentation “Pollen exposure levels on horse grounds and the urban human environment. A collaborative study with the Austrian Pollen Information Service.” Meeting of the Scientific Advisory Board of the interuniversity Messerli Research Institute, Vienna, Austria

December 30, 2018 Poster presentation “Qualitative and quantitative comparison of pollen allergen exposure between horses and humans. A collaborative study with the Austrian Pollen Information Service.” – Retreat of the interuniversity Messerli Research Institute, 2018 Baden, Austria

November 12, 2018 Abstract “Why do IgE profiles differ in humans and horses? Analyzing pollen allergen exposure on pastures and paddocks in collaboration with the Austrian Pollen Information Service.” NEXT GENERATION ÖGAIng of the Austrian Society of Allergy and Clinical Immunology, November 12, 2018, Vienna, Austria

September 17, 2018 Poster presentation und Running Slide Show “Difference in pollen allergen exposure between horses and humans? A collaborative study with the Austrian Pollen Information Service.” at the 9th Retreat of the Center for Pathophysiology, Infectiology and Immunology (CePII), Medical University Vienna, Vienna, Austria

September 2-5, 2018 Poster presentation “Qualitative and quantitative comparison of pollen allergen exposure between horses and humans. A collaborative study with the Austrian Pollen Information Service.“ at the 5th European Congress of Immunology, Amsterdam, The Netherlands

Lectures

September 3, 2019 Lecture “nutrition in dogs” within the IMHAI Master program

October 31, 2018 Invited lecture at the Comparative Medicine-Minisymposium „Pollen im Anflug: Gefahr für Mensch & Tier?“ – Vienna, Austria

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8. LIST OF FIGURES

Figure 14: Western blot of all seasons at location C. Bet v 1…positive control, M…middle bloomers, G…grass season, L…late bloomers, E…early bloomers; 1…pasture, 2…paddock

9. LIST OF TABLES

Table 06: Protein concentration of each sample determined by the NanoPhotometer® P 300. E… early bloomers, M… middle bloomers, G… grass season, L… late bloomers; 1… pasture, 2… paddock

10. ABBREVIATIONS

µg: microgram

µl: microliter

APCs: antigen presenting cells

APIS: Austrian Pollen Information Service

BSA: bovine serum albumin

COPD: Chronic obstructive pulmonary disease

ddH_2O: distilled water

DTH: delayed – type hypersensitivity

h: hour

HRP: horse reddish peroxide

IC: immunocomplex

Ig: immunoglobulin

IL: interleukins

kDa: kilo Dalton

l: litre

mg: milligram

MHC II: major histocompatibility complex II

min: minute

ml: millilitre

nm: nanometre

PBS: Phosphate Buffered Saline

RAO: Recurrent airway obstruction

rpm: rounds per minute

RT: room temperature

SDS – PAGE: Sodium dodecyl sulphate–polyacrylamide gel electrophoresis

sec: second

T: Tween

Th2: T-helper cells 2

TLR: toll like receptors

11. SUPPLEMENTARY MATERIAL

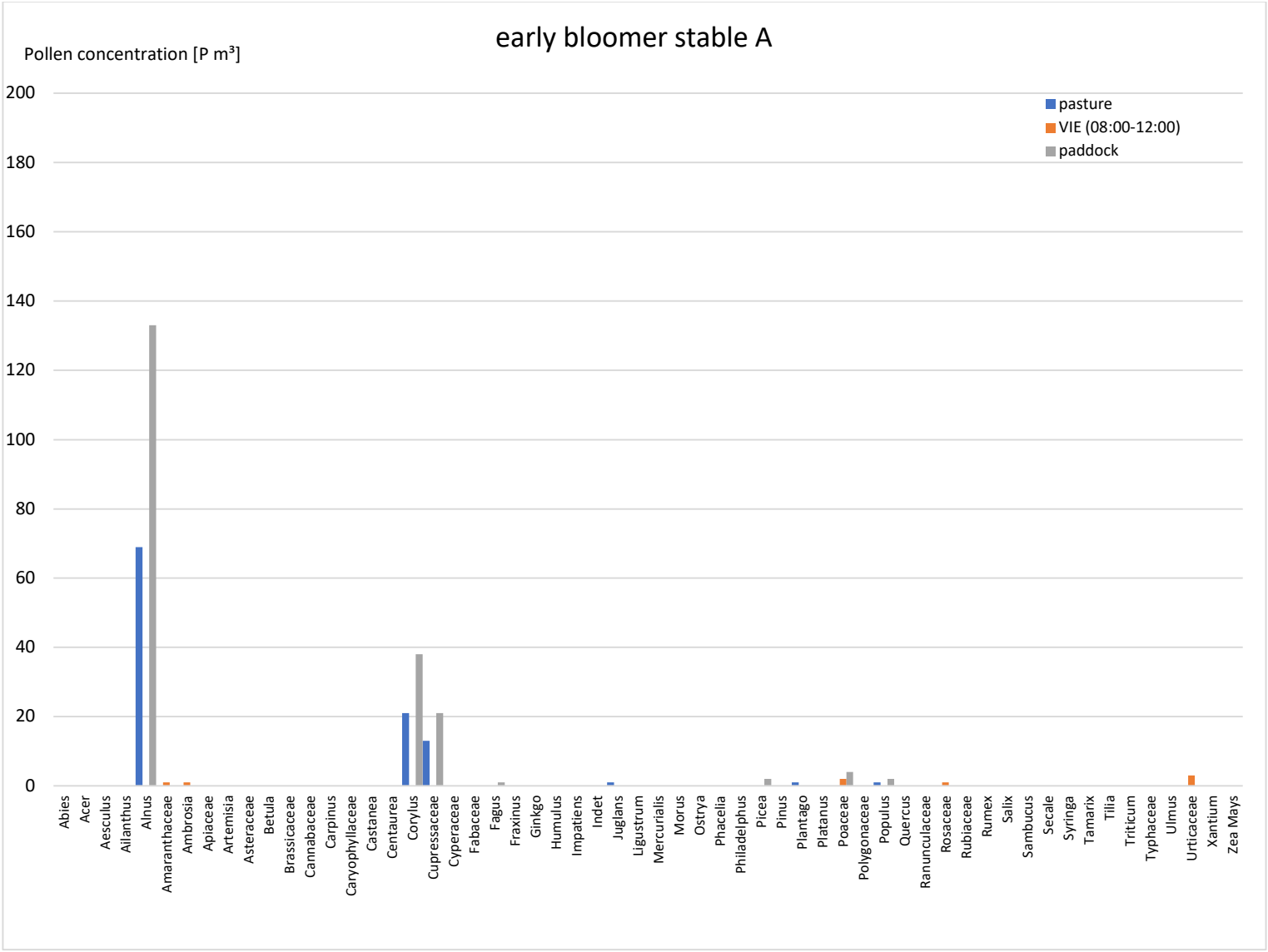

Figure 01: Pollen distribution in the early bloomer season found at location A

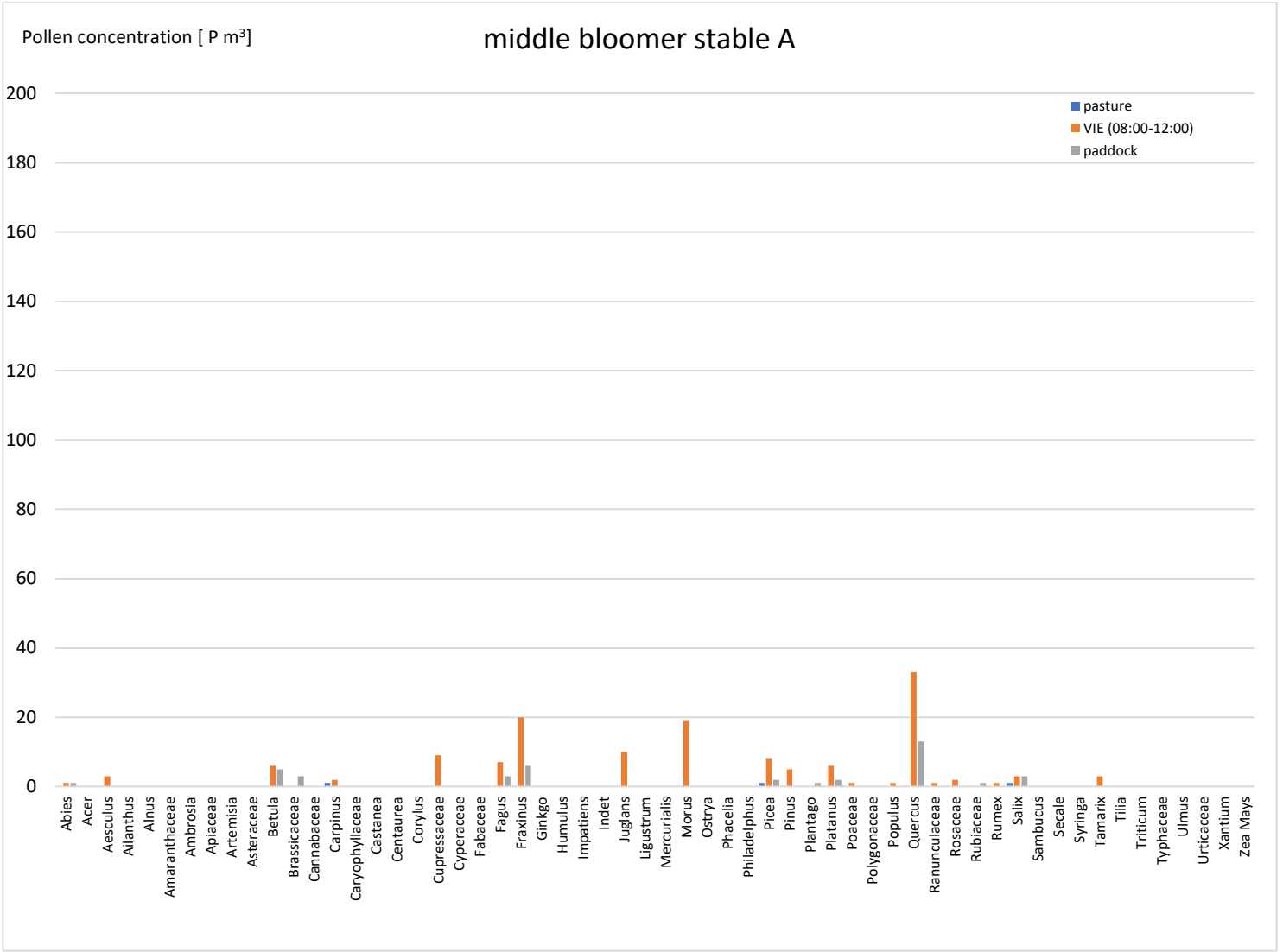

Figure 02: Pollen distribution in the middle bloomer season found at location A

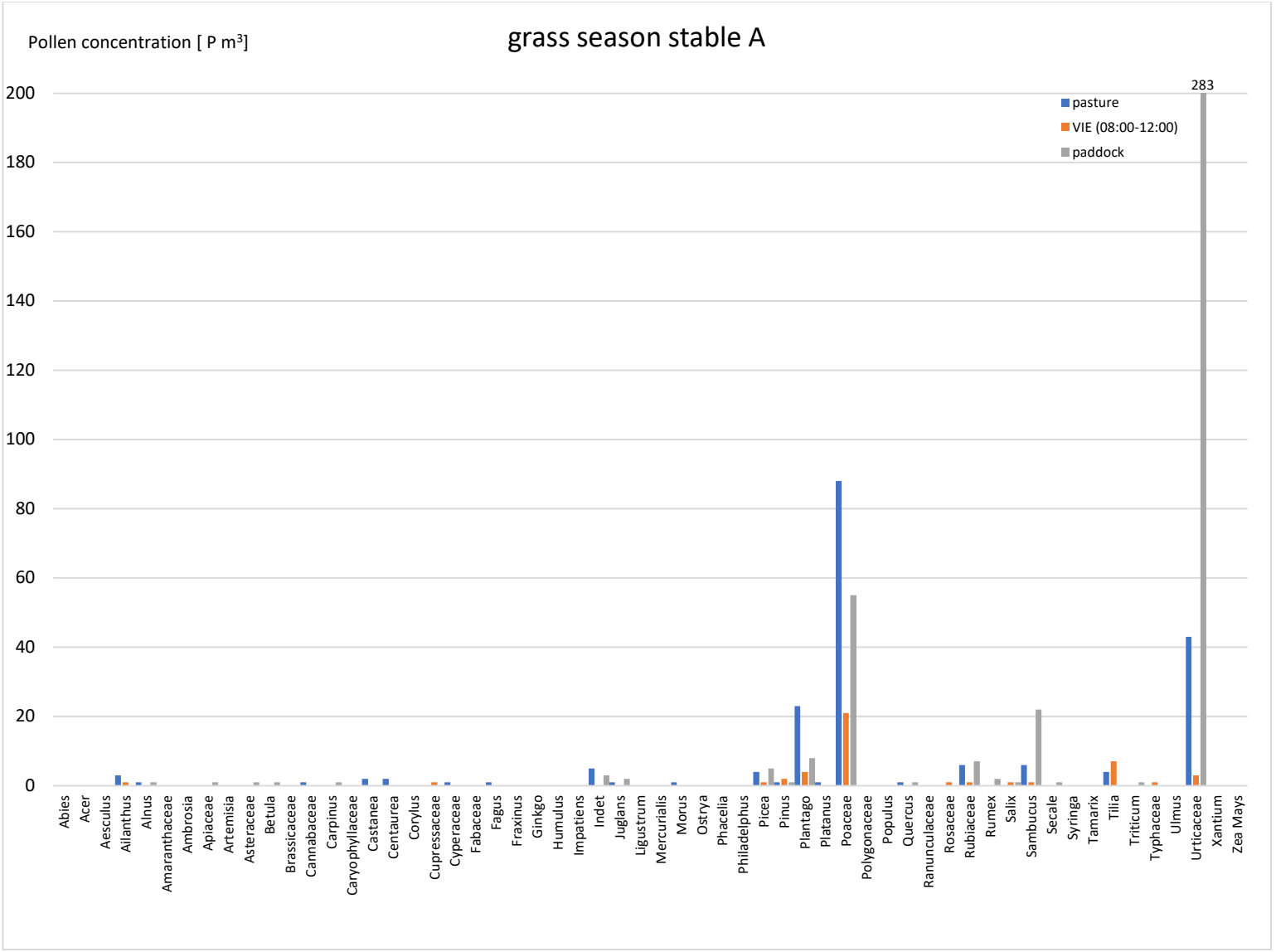

Figure 03: Pollen distribution in the grass season found at location A

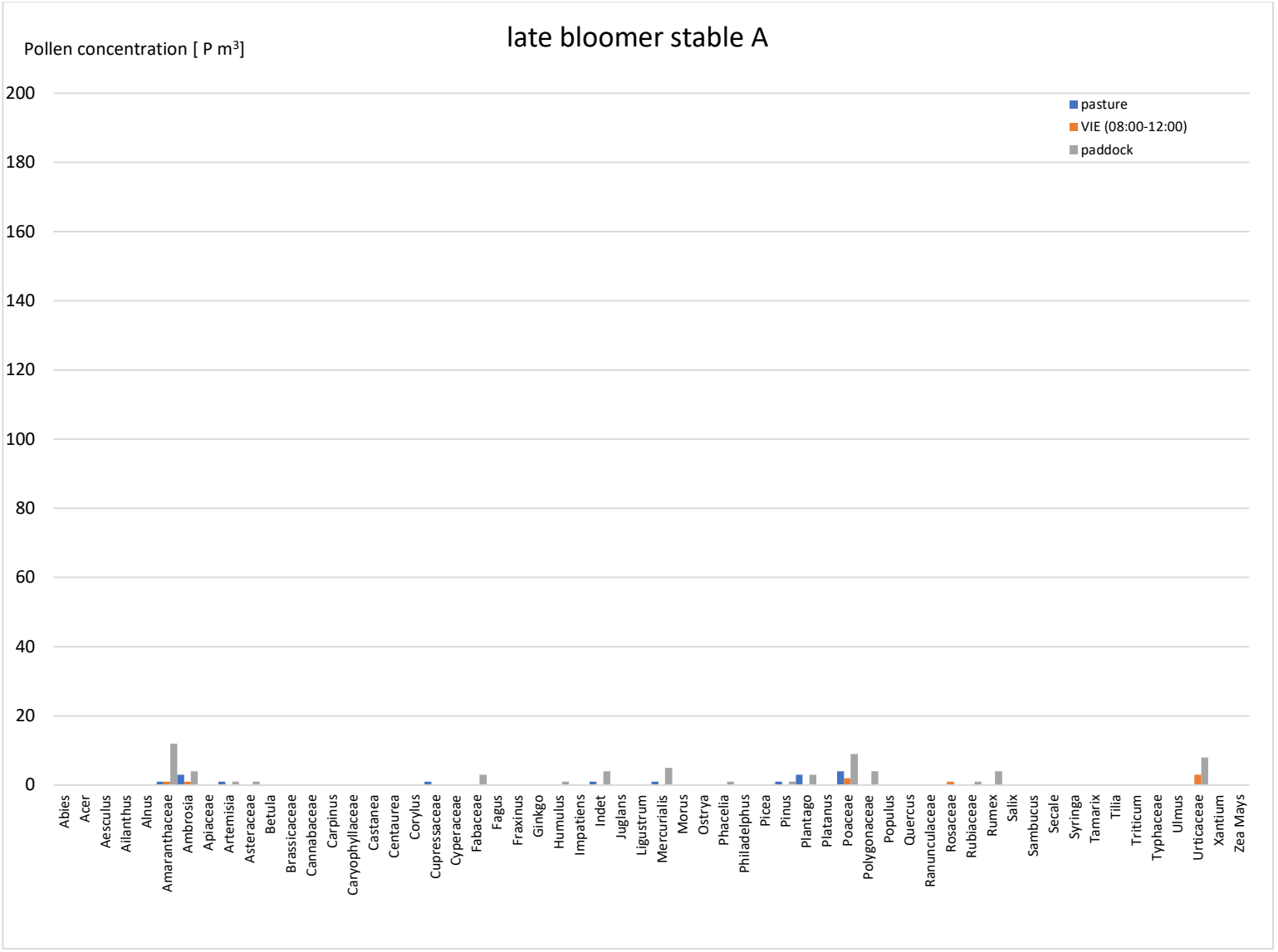

Figure 04: Pollen distribution in the late bloomer season found at location A

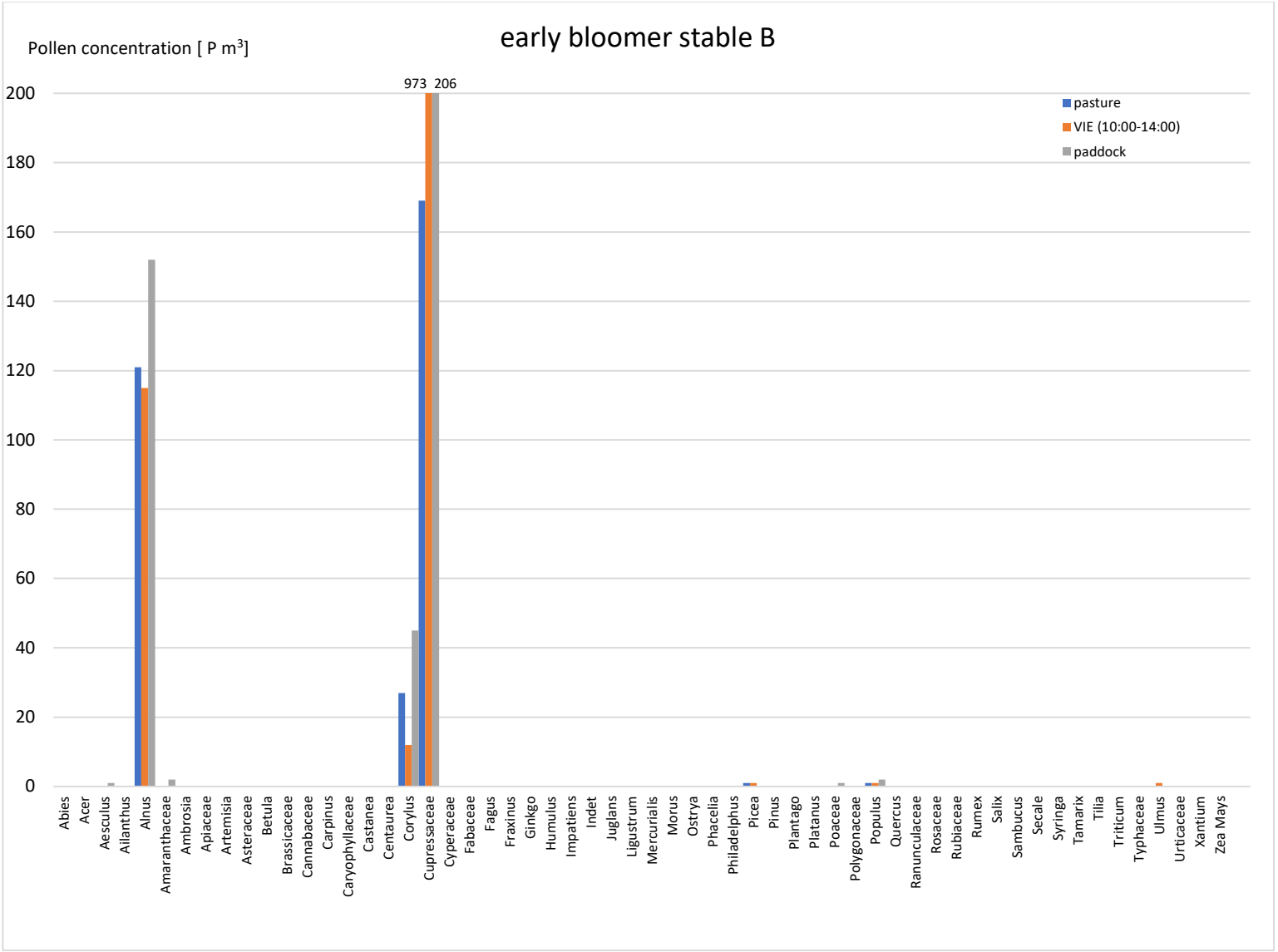

Figure 05: Pollen distribution in the early bloomer season found at location B

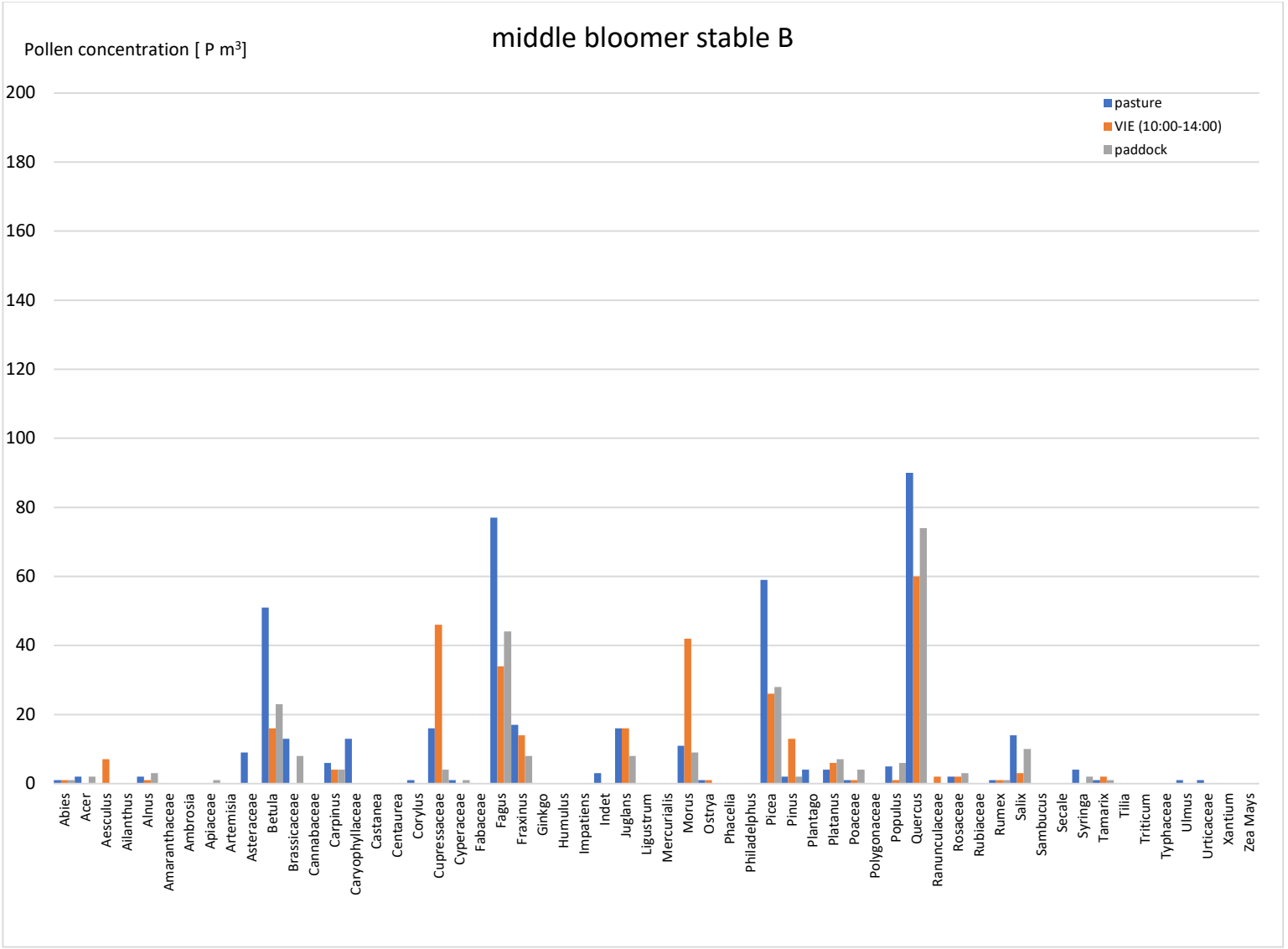

Figure 06: Pollen distribution in the middle bloomer season found at location B

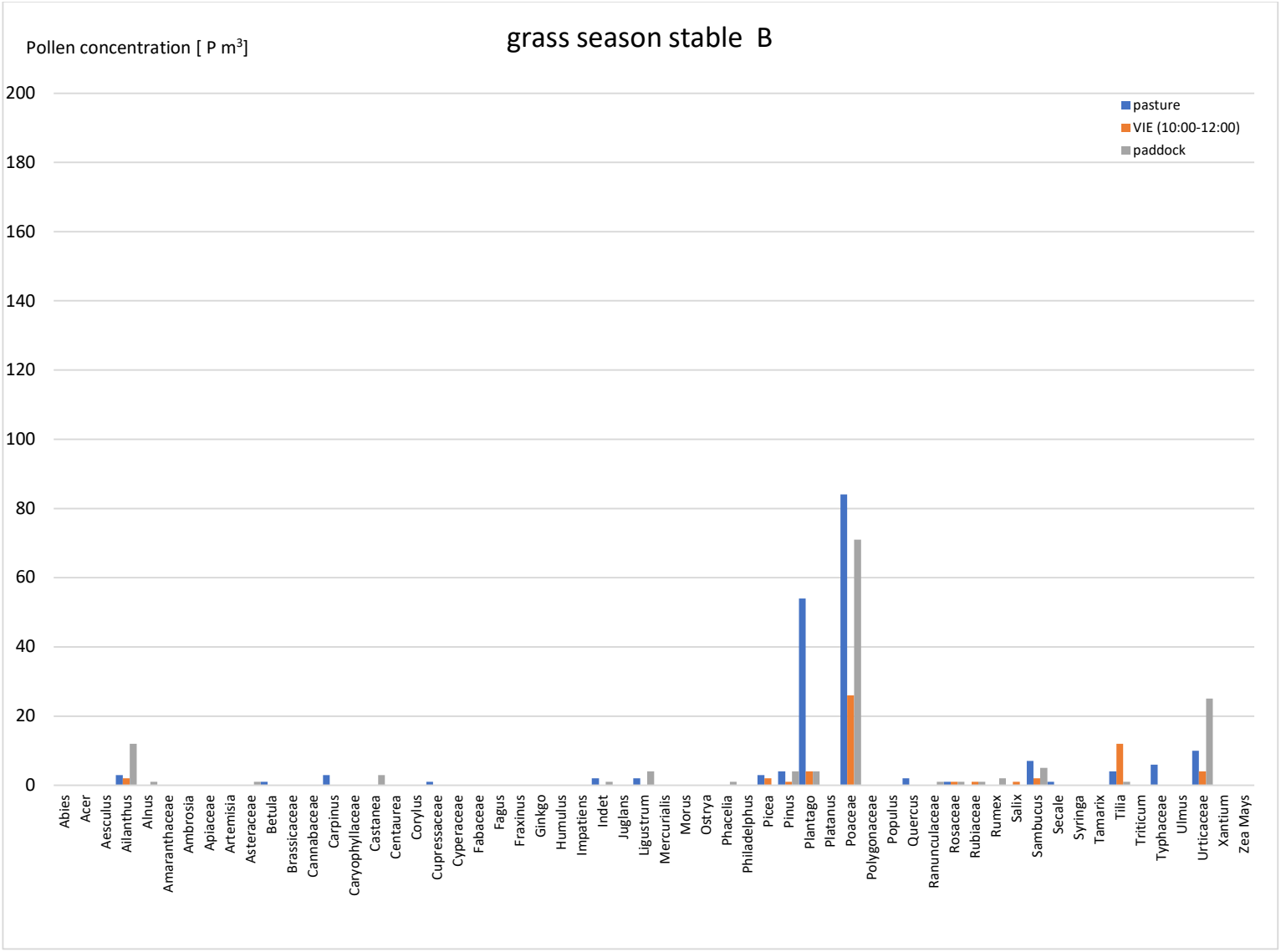

Figure 07: Pollen distribution in the grass season found at location B

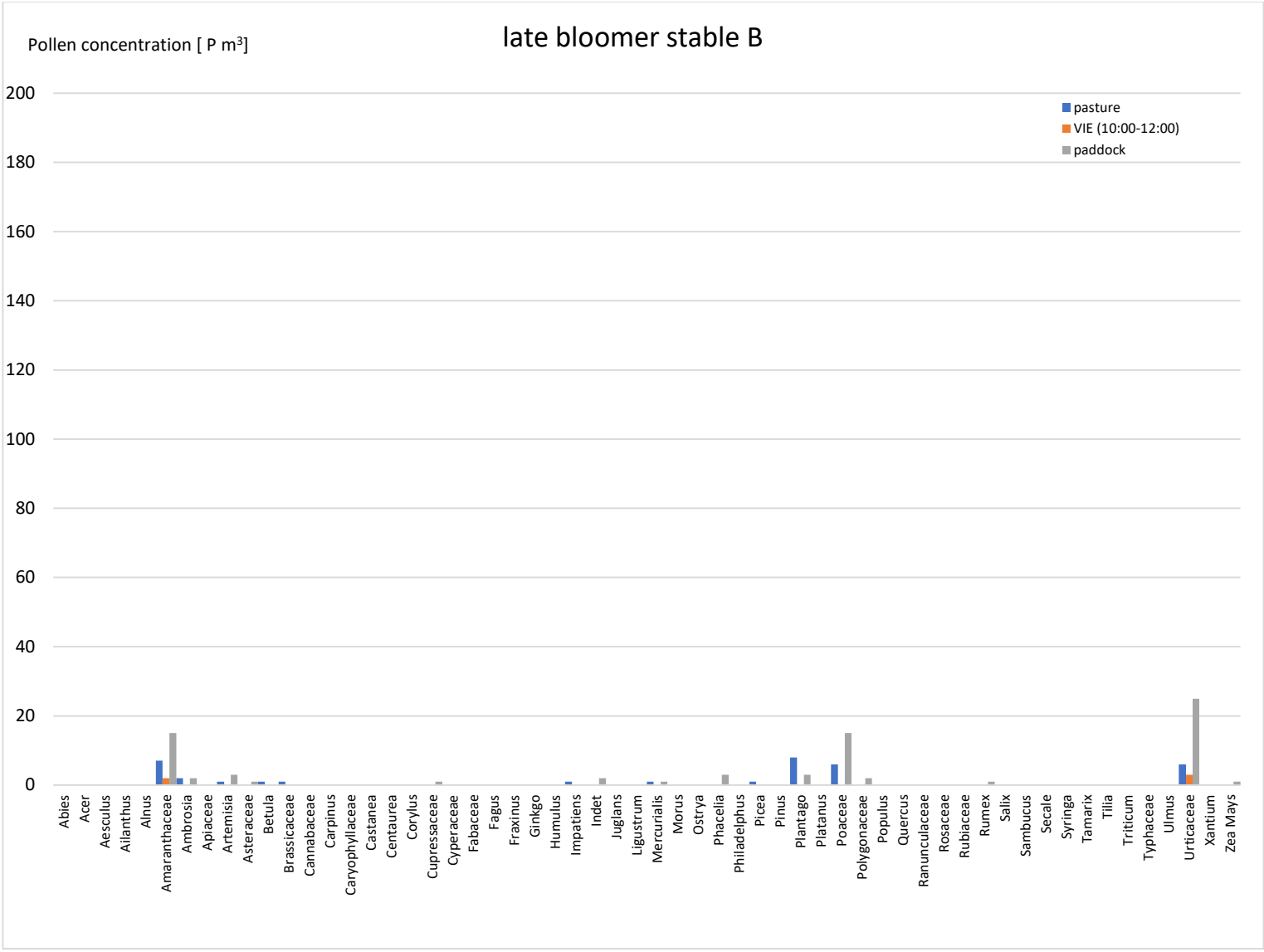

Figure 08: Pollen distribution in the late bloomer season found at location B

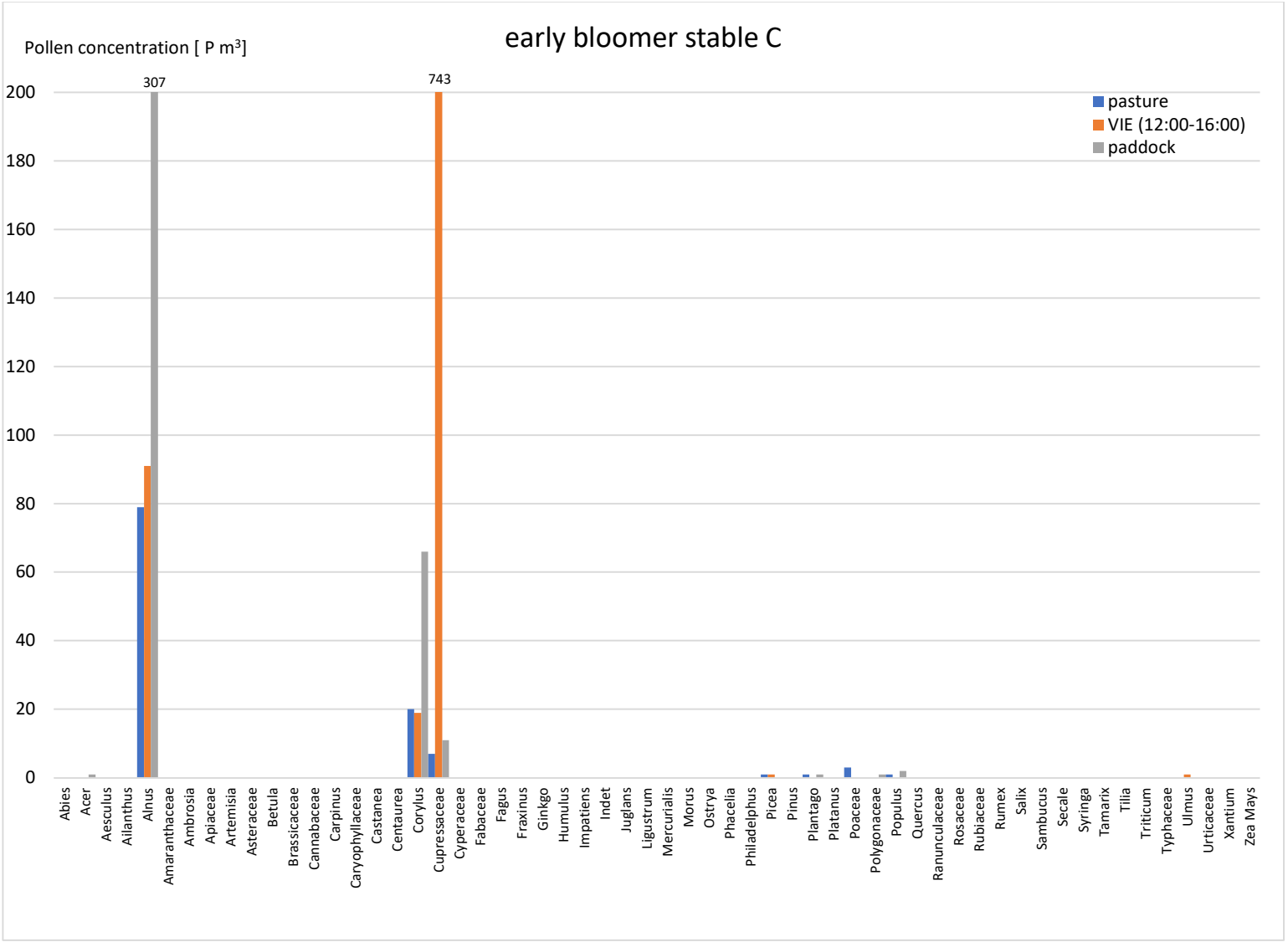

Figure 09: Pollen distribution in the early bloomer season found at location C

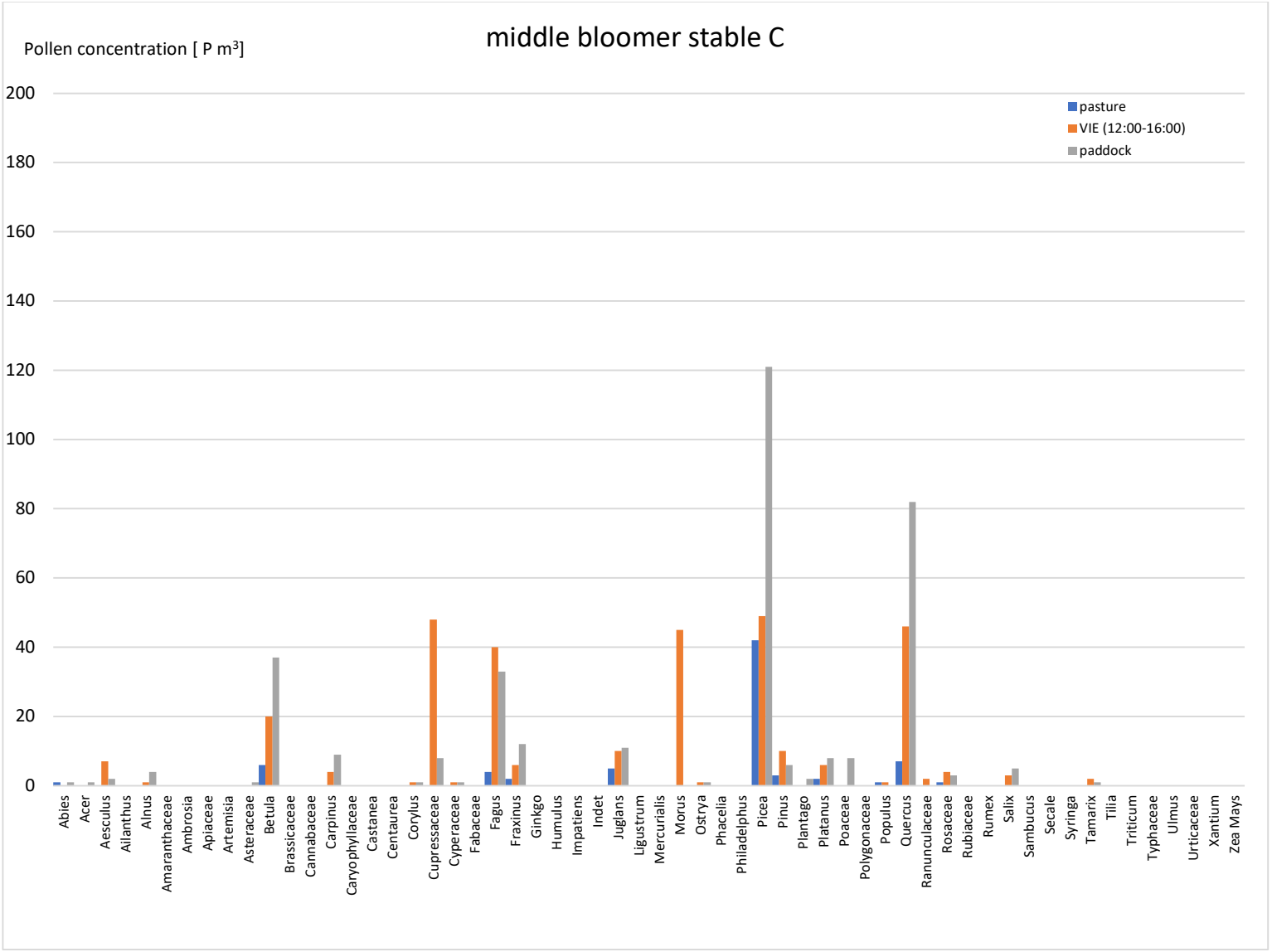

Figure 10: Pollen distribution in the middle bloomer season found at location C

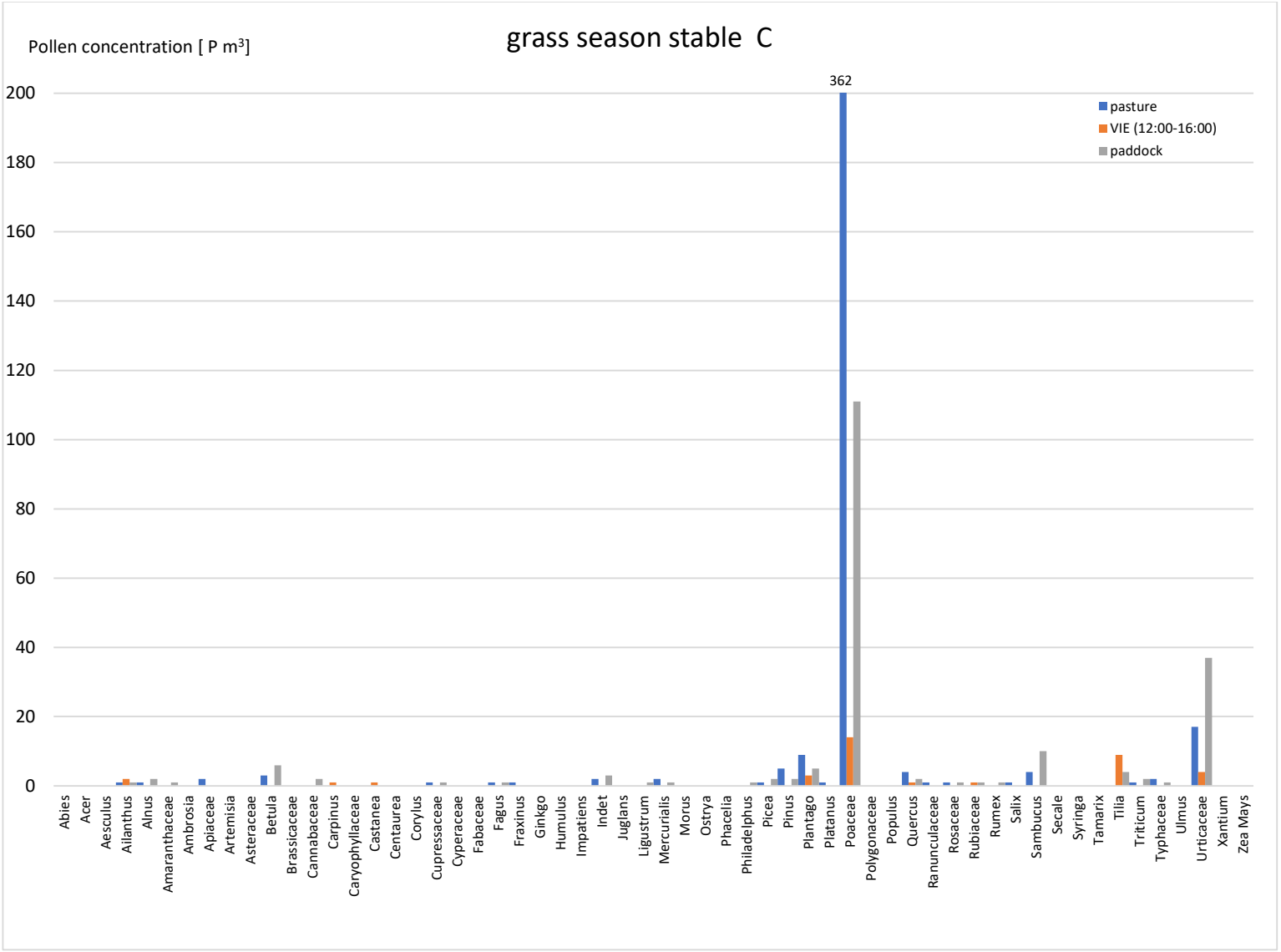

Figure 11: Pollen distribution in the grass season found at location C

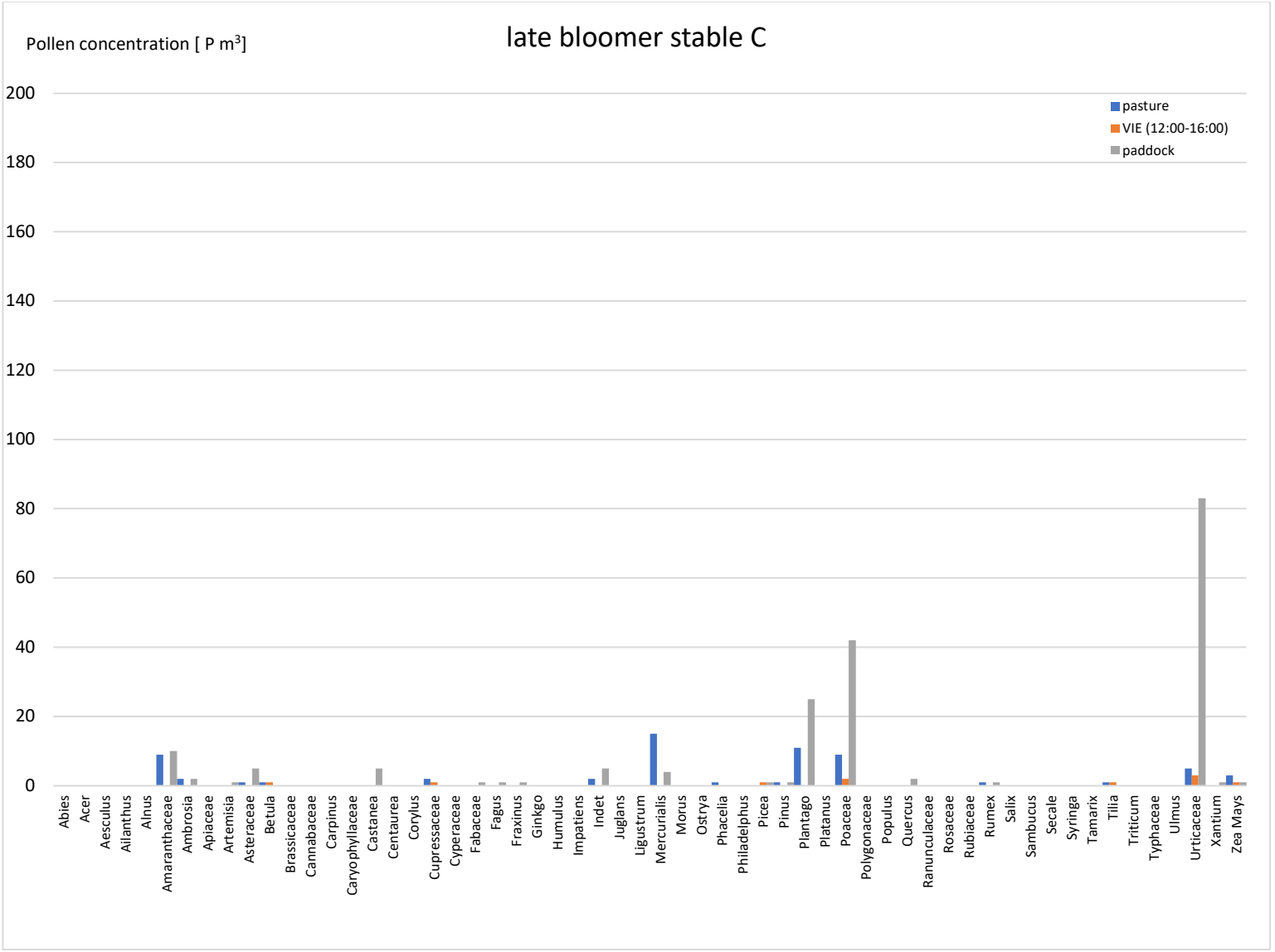

Figure 12: Pollen distribution in the late bloomer season found at location C

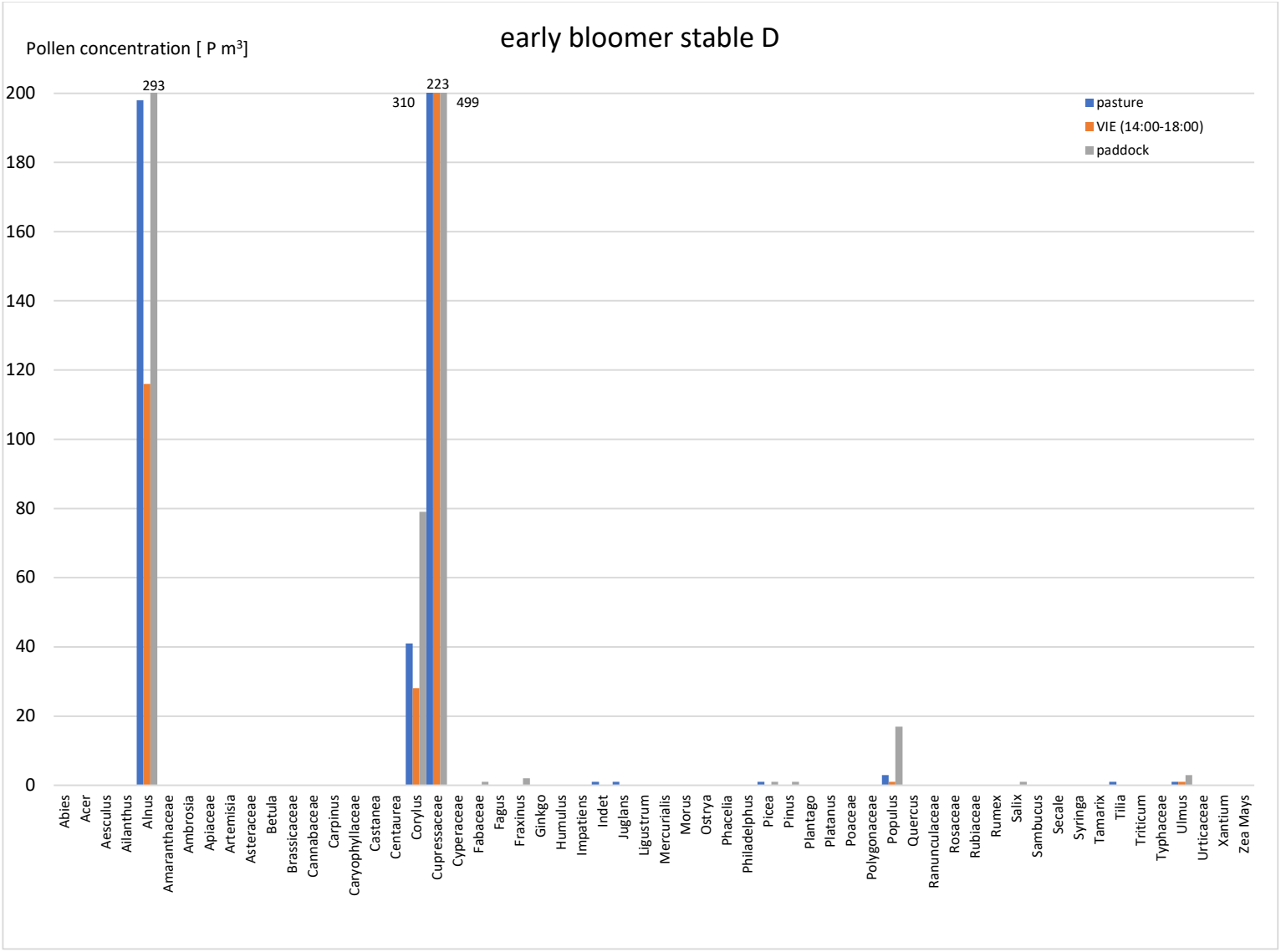

Figure 13: Pollen distribution in the early bloomer season found at location D

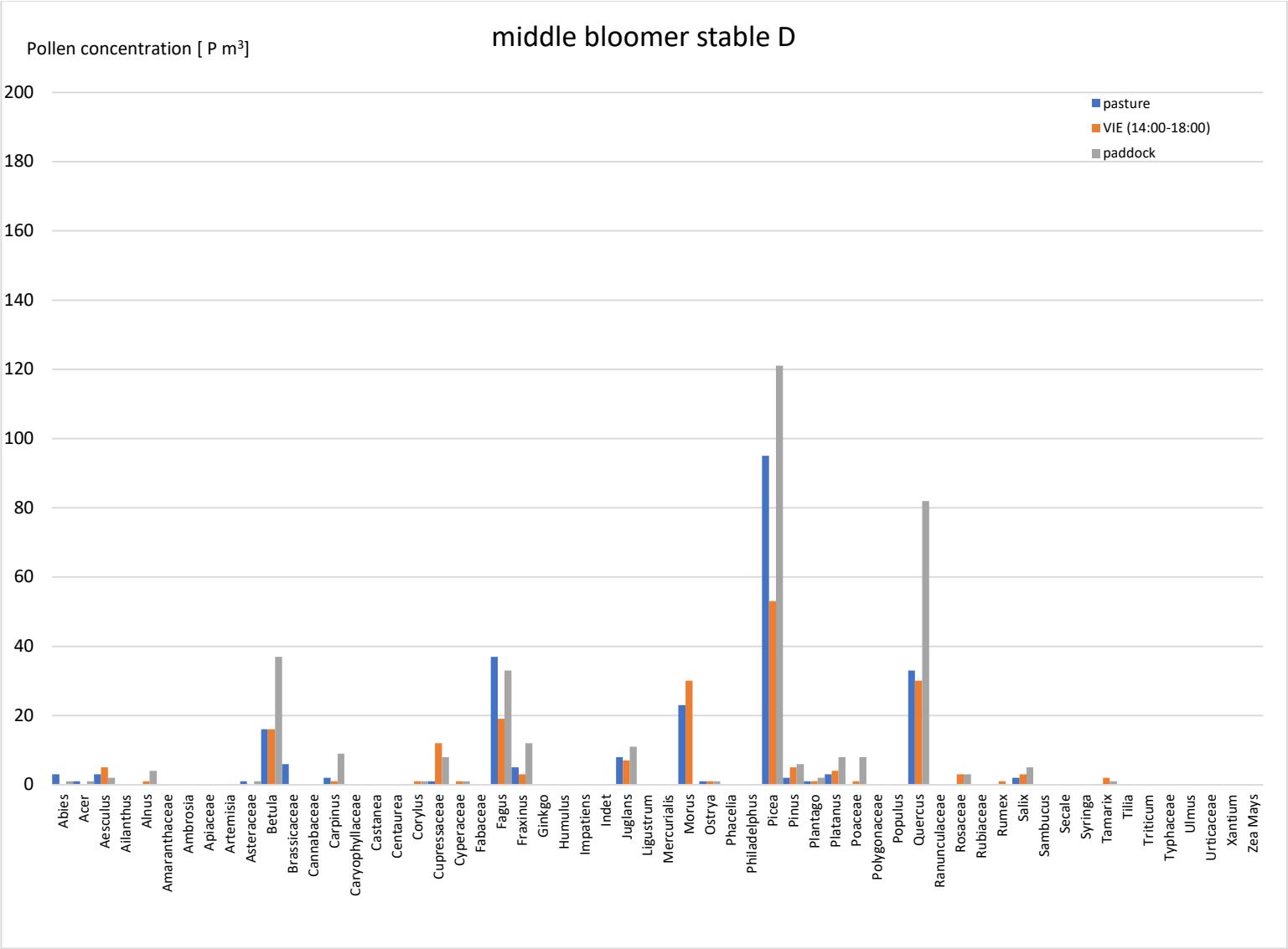

Figure 14: Pollen distribution in the middle bloomer season found at location D

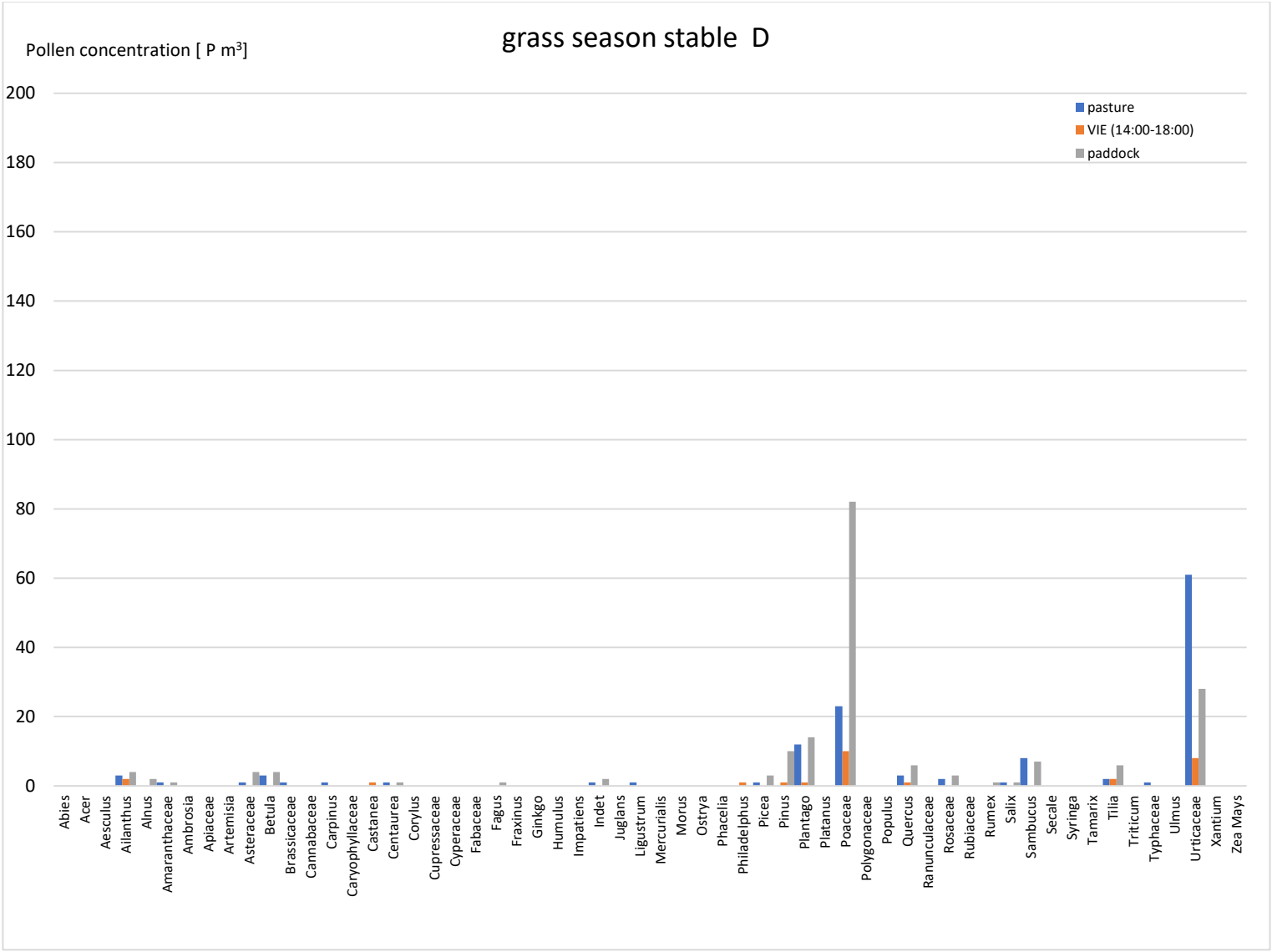

Figure 15: Pollen distribution in the grass season found at location D

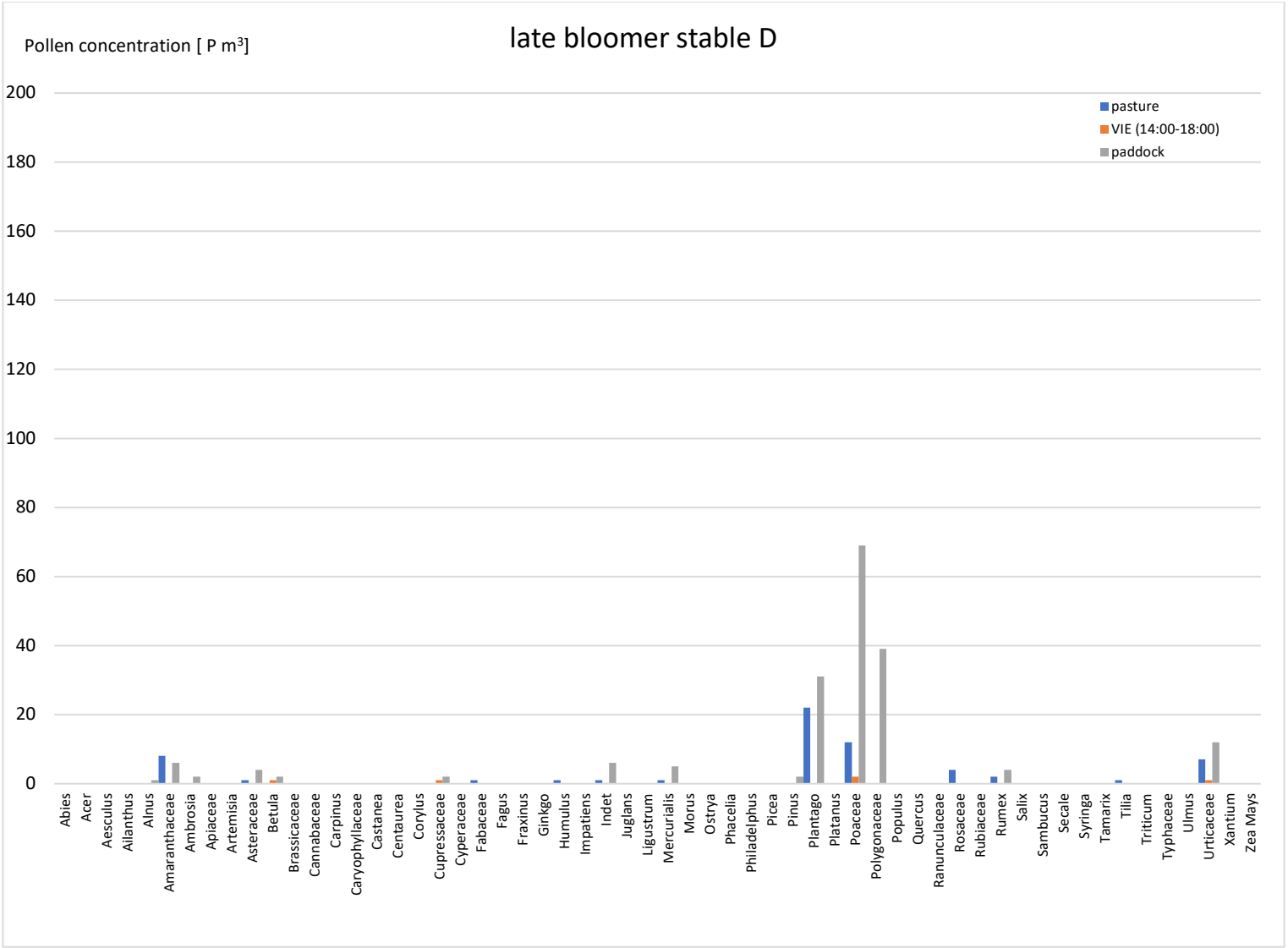

Figure 16: Pollen distribution in the late bloomer season found at location D

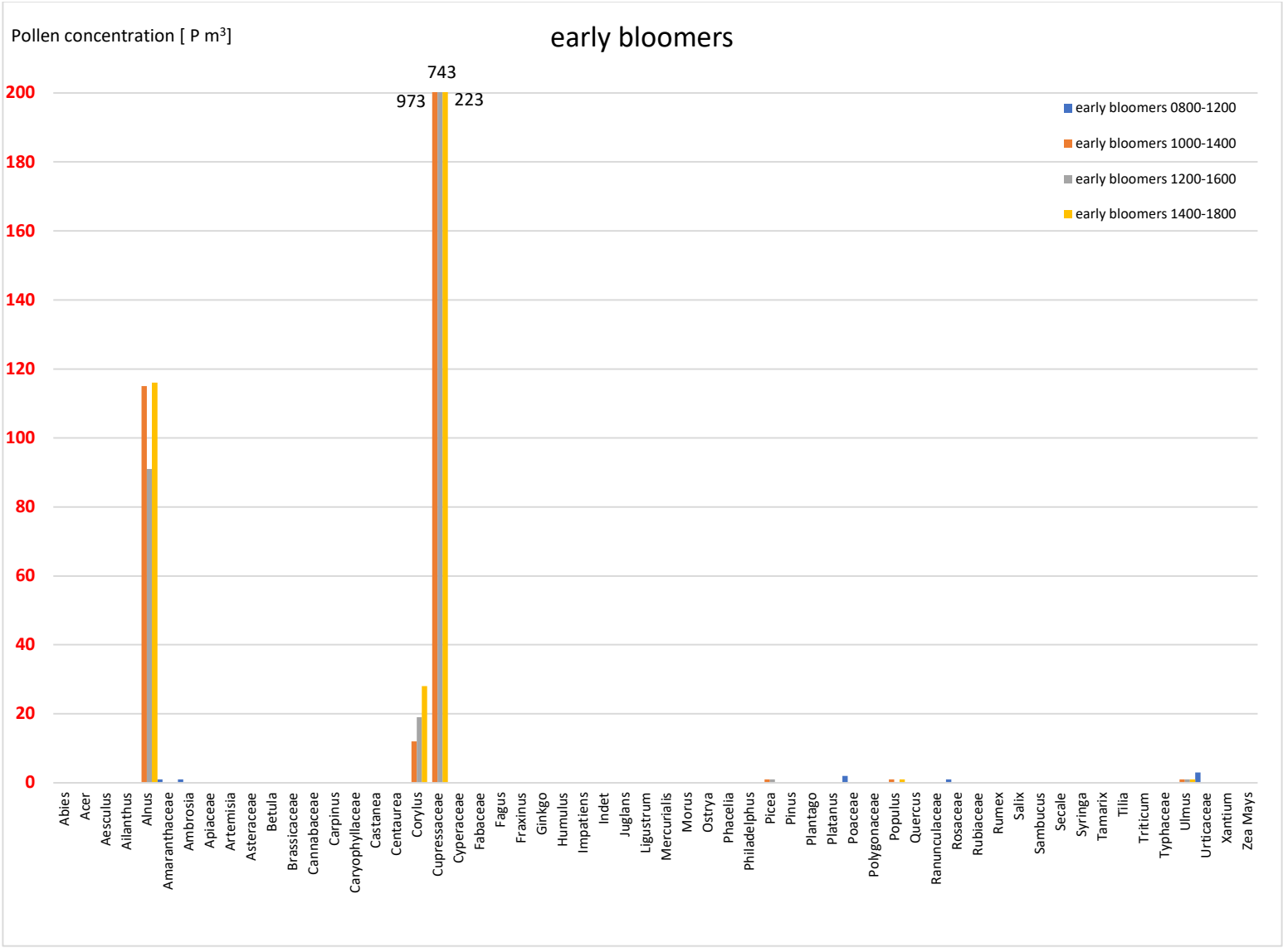

Figure 17: Pollen distribution in the early bloomer season at Vienna measured at different times a day.

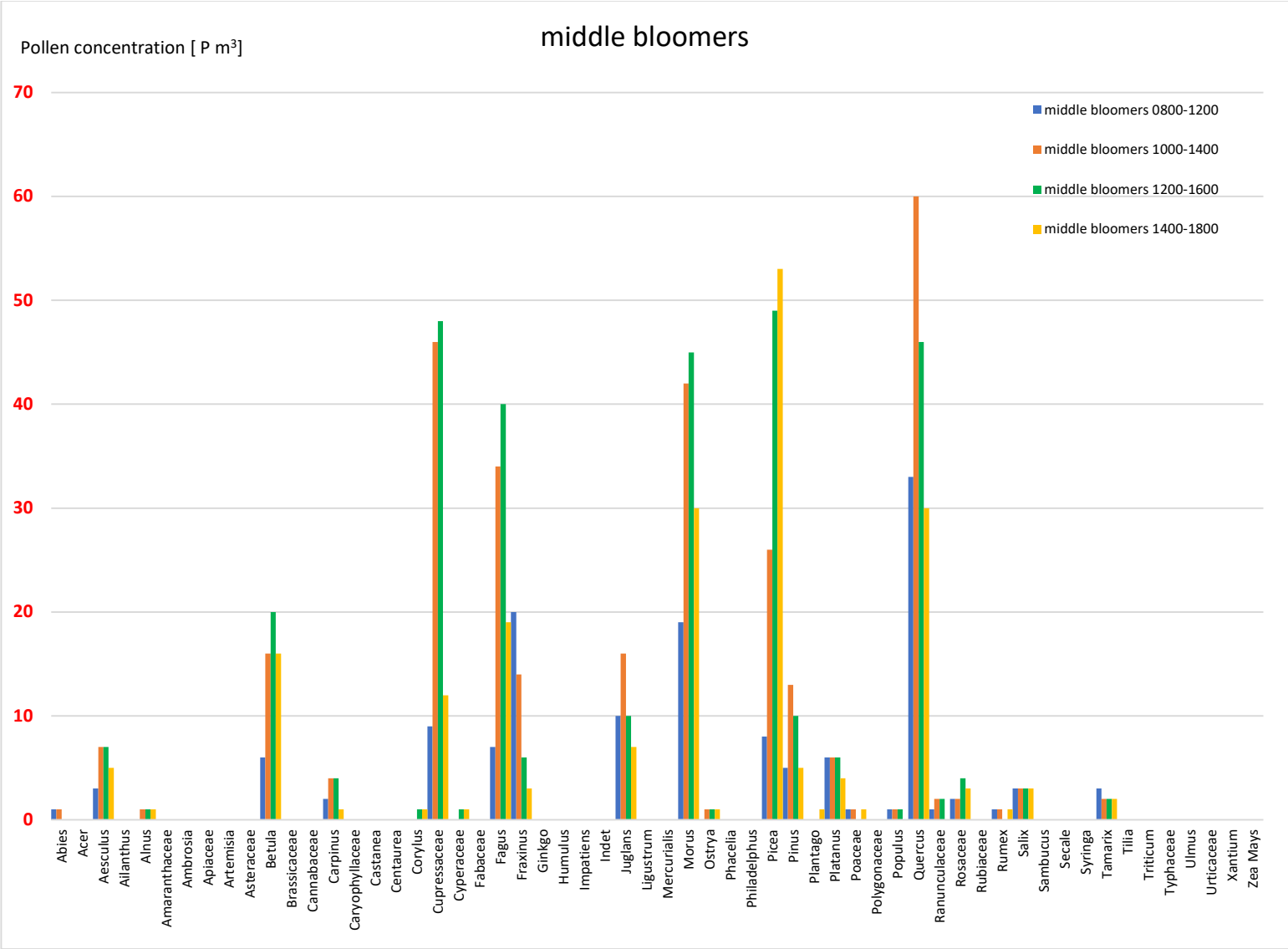

Figure 18: Pollen distribution in the middle bloomer season at Vienna measured at different times a day.

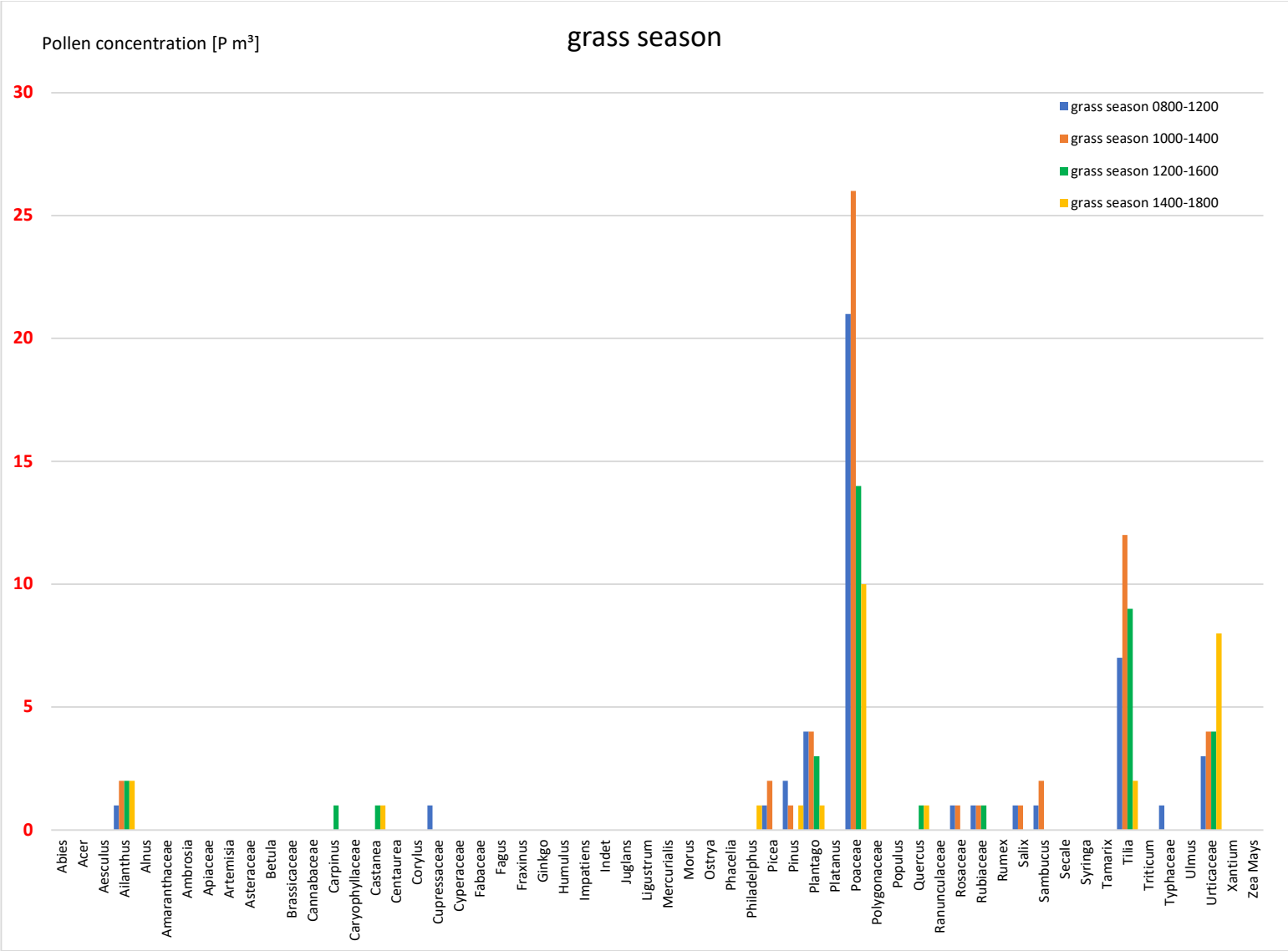

Figure 19: Pollen distribution in the grass season at Vienna measured at different times a day.

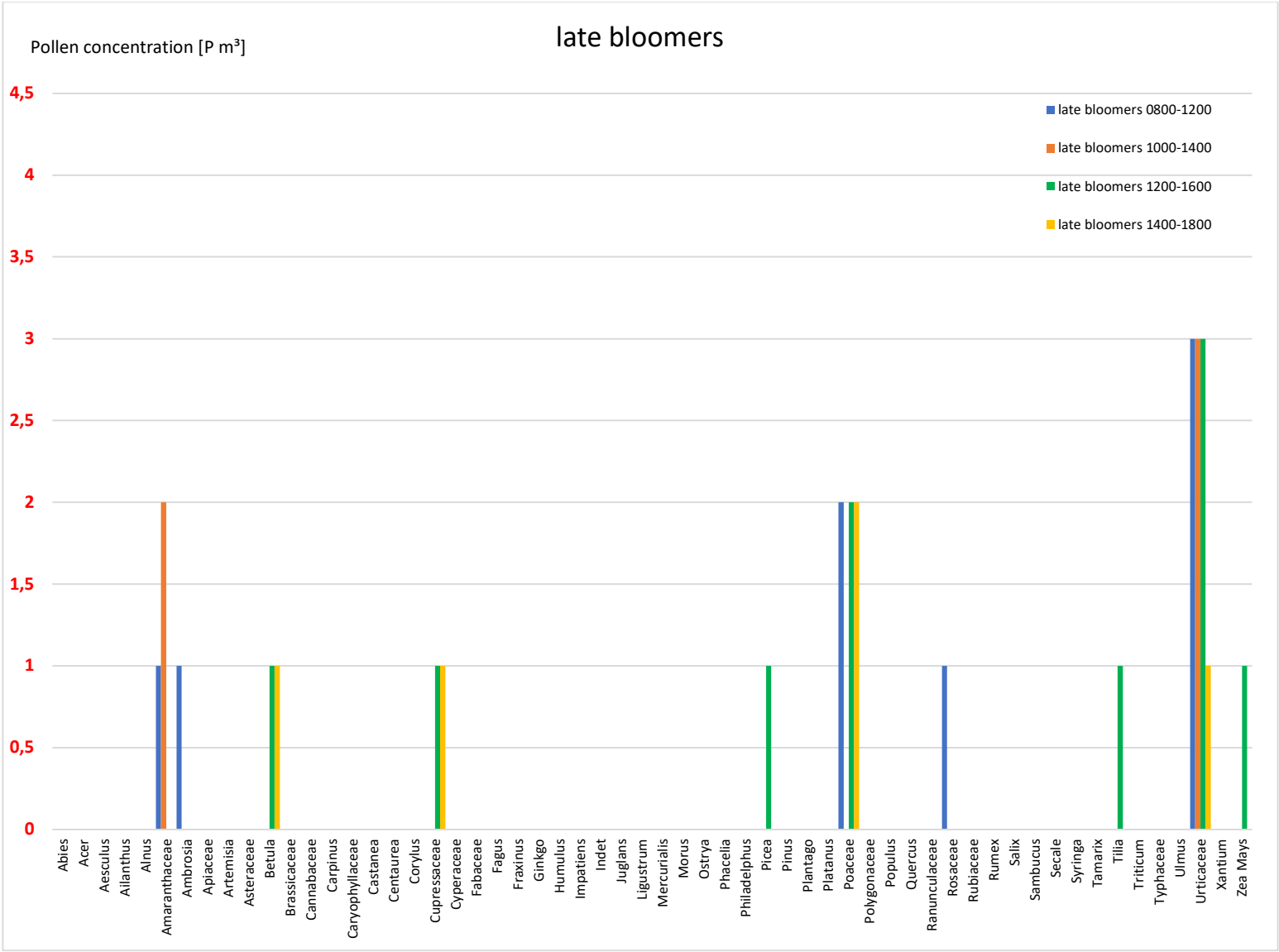

Figure 20: Pollen distribution in the late bloomer season at Vienna measured at different times a day.