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Master Thesis

Parasitoids and pathogens of the gypsy moth, *Lymantria dispar* L. (Lep., Erebidae), during retrogradation at an outbreak site in Lower Austria

Submitted by

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I Abstract

In 2018, a population outbreak of the gypsy moth, *Lymantria dispar* L. (Lep., Erebidae), was observed in Lower Austria, resulting in total defoliation of an oak forest in the summers of 2018 and 2019. In 2020, the population density was still high, but the population was expected to decline. The present work investigated the role of natural enemies in the collapse of the outbreak population.

In total, 20 egg masses, 680 larvae, and 12 pupae of *L. dispar* were collected in the field between May and July 2020 and reared until emergence of adult moths or death. Causes of mortality were determined based on emerging parasitoids and phase contrast microscopy of non-parasitized cadavers. Stage-specific mortality rates were determined for eggs, all larval instars (L1-L6), and pupae. Mortality was caused by seven parasitoid and three pathogen species. Starting from a high density in spring, the gypsy moth population declined to a negligible level until summer.

The egg parasitoid *Anastatus disparis* (Hym., Eupelmidae) emerged from 19 % of the *L. dispar* eggs. In gypsy moth larvae, parasitoids caused stage-specific mortality rates of 15 % (L1) to 61 % (L6). The dominant parasitoid of young and middle-aged larvae was *Glyptapanteles porthetriae* (Hym., Braconidae) (L1-L4: 10-36 % stage-specific parasitization). Mature larvae were mainly parasitized by *Blepharipa pratensis* (Dip., Tachinidae) and *Parasetigena silvestris* (Dip., Tachinidae), which each emerged from 26 % of larvae collected in the final instars (L5+L6). Further parasitoid species observed were *Hyposoter tricoloripes* (Hym., Ichneumonidae), *Glyptapanteles liparidis* (Hym., Braconidae) and *Cotesia* sp. (Hym., Braconidae).

Pathogens caused stage-specific mortality rates of 11 % (L3) to 41 % (L1). The Nuclear Polyhedrosis Virus (*Ld*NPV) was the dominant pathogen in all instars (7-34 % stage-specific mortality). *Entomophaga maimaiga* – an entomopathogenic fungus detected for the first time in Austria in 2019 – caused low mortality rates in older larvae (L4-L6: 4-5 % stage-specific mortality). The microsporidium *Endoreticulatus schubergi* and unidentified fungal species caused low mortality rates.

II Kurzfassung

Im Jahr 2018 wurde in Niederösterreich eine Massenvermehrung des Schwammspinners, *Lymantria dispar* L. (Lep., Erebidae), beobachtet, die zum Kahlfraß des betroffenen Eichenwaldes in den Sommern 2018 und 2019 führte. Für 2020 wurde eine weiterhin hohe, jedoch abnehmende Populationsdichte erwartet. Die vorliegende Arbeit untersuchte die Rolle von natürlichen Gegenspielern bei der Beendigung der Massenvermehrung.

Insgesamt wurden 20 Eigelege, 680 Raupen und 12 Puppen des Schwammspinners zwischen Mai und Juli 2020 im Freiland gesammelt und bis zum Schlupf adulter Falter bzw. bis zum Tod aufgezogen. Die Todesursache wurde anhand sich entwickelnder Parasitoide und durch Phasenkontrastmikroskopie nichtparasitierter Kadaver ermittelt. Stadienspezifische Mortalitätsraten wuden für Eier, alle Raupenstadien (L1-L6) und Puppen erhoben. Sieben Parasitoide und drei Pathogenarten wurden als Todesursachen nachgewiesen. Ausgehend von einer hohen Dichte im Frühling, brach die Schwammspinnerpopulation bis zum Sommer nahezu vollständig zusammen.

Der Eiparasitoid *Anastatus disparis* (Hym., Eupelmidae) entwickelte sich aus 19 % der *L. dispar* Eier. Unter Raupen verursachten Parasitoide stadienspezifische Mortalitätsraten von 15 % (L1) bis 61 % (L6). Der dominante Parasitoid junger und mittlerer Raupenstadien war *Glyptapanteles porthetriae* (Hym., Braconidae) (L1-L4: 10-36 % stadienspezifische Parasitierungsraten). Altraupen wurden hauptsächlich durch *Blepharipa pratensis* (Dip., Tachinidae) und *Parasetigena silvestris* (Dip., Tachinidae) parasitiert, die sich jeweils aus 26 % der in den finalen Stadien gesammelten Raupen (L5+L6) entwickelten. Weiters wurden die Parasitoidenarten *Hyposoter tricoloripes* (Hym., Ichneumonidae), *Glyptapanteles liparidis* (Hym., Braconidae) und *Cotesia* sp. (Hym., Braconidae) beobachtet.

Pathogene verursachten stadienspezifische Mortalitätsraten von 11 % (L3) bis 41 % (L1). Die Kernpolyedervirose *Ld*NPV dominierte unter den Pathogenen (7-34 % stadienspezifische Mortalität). *Entomophaga maimaiga* – ein 2019 erstmals in Österreich nachgewiesenes Pathogen – verursachte niedrige Mortalitätsraten unter älteren Raupen (L4-L6: 4-5 % stadienspezifische Mortalität). Die Mikrosporidie *Endoreticulatus schubergi* und nicht identifizierte Pilzarten verursachten niedrige Mortalitätsraten.

III Acknowledgment

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IV Statutory declaration

I hereby declare that I am the sole author of this work. No assistance other than that which is permitted has been used. Ideas and quotes taken directly or indirectly from other sources are identified as such. This written work has not yet been submitted in any part.

Thomas Zankl

V Content

1 Introduction and state of knowledge

1.1 Motivation and goals of this thesis

The gypsy moth, *Lymantria dispar* (Linnaeus) (Lep., Erebidae), is a serious defoliating forest pest with a very divers complex of natural enemies in its native area of distribution, including numerous parasitoids (ŽIKIĆ et al., 2017), predators (SMITH and LAUTENSCHLAGER, 1978; GSCHWANTNER et al., 2002), and pathogens (NOVOTNÝ, 1989; WEISER, 1998). Several field studies investigated the composition of the natural enemy complex of the gypsy moth in the past, which highlighted their important impact on the population dynamics of *L. dispar*. An overview of studies in Austria and neighbouring countries is given in **Table 1**. In Austria, studies were conducted in the 1970's, 1990's, and most recently in 2004. The focus of preceding studies was predominantly on parasitoids.

Table 1: Overview of studies on the natural enemy complex of *Lymantria dispar* in Austria and neighbouring countries, considering literature published in English and German.

Author(s)	Study year(s)	Country/Region	Focus
FUESTER et al., 1983	1974-75	Austria, Germany	Parasitoids
PSCHORN-WALCHER, 1977	1978	Austria	Parasitoids
EICHHORN, 1996	1979	Austria	Parasitoids
HOCH, 1995	1993-94	Austria	Parasitoids, Pathogens
SCHOPF and HOCH, 1997	1993-95	Austria	Parasitoids
GSCHWANTNER et al., 2002	1998	Austria	Predators
KALBACHER, 2008	2003-04	Austria	Parasitoids
BURGESS and CROSSMAN, 1929	1905-27	Europe	Parasitoids
ČAPEK, 1988	1963-74	Slovakia	Parasitoids
MAIER, 1990	1984-86	Germany	Parasitoids
ZÚBRIK and NOVOTNÝ, 1997	1991-95	Slovakia	Parasitoids
WERMELINGER, 1995	1992-93	Switzerland	Predators
BATHON, 1993	1993	Germany	Parasitoids
HOCH et al., 2001	1993-96	Austria, Slovakia	Parasitoids, Pathogens
TURCÁNI et al., 2001	1991-99	Slovakia	Parasitoids, Pathogens, Predators
CAMERINI, 2009	1997-2007	Italy	Parasitoids
CONTARINI et al., 2013	2010-11	Italy	Parasitoids, Pathogens
ALALOUNI et al., 2014	2011-12	Germany	Parasitoids, Pathogens

From 2005, no mentionable damage by *L. dispar* occurred in Austria until local population outbreaks were observed in 2018. In 2019, *Entomophaga maimaiga* – an introduced entomopathogen of the gypsy moth that is currently spreading in Europe – was detected for the first time in Austria at two outbreak sites (Eggenburg and Ebergassing) (HOCH et al., 2019). In Eggenburg, total defoliation of an oak forest by gypsy moth larvae was observed in 2018 and 2019. For 2020, still high population density was expected, but the population was assumed to reach the retrogradation period.

These circumstances in Eggenburg in 2020, offered an opportunity to conduct an observational study on the natural enemy complex of *L. dispar* that is markedly differing from preceding Austrian studies in two aspects. The first aspect is temporospatial, since all preceding study sites were in Burgenland, and more than 40 years have passed since the first studies, within a period of intensive climatic changes (FORMAYER et al., 2009). Secondly, *E. maimaiga* is not only known for its ability to cause extensive epizootics in gypsy moth populations, but also as an important competitor of other members of the natural enemy complex (TABAKOVIĆ-TOŠIĆ et al., 2014; HAJEK et al., 2015). The present thesis shall complement previous works

on the impact of parasitoids and pathogens as mortality factors in Austrian gypsy moth field populations, with emphasis to these two aspects. For this purpose, eggs, larvae, and pupae of *L. dispar* were collected stage-specifically and reared until death or adult emergence, to measure apparent parasitism and pathogen mortality.

1.2 Host plants and generation cycle of *Lymantria dispar*

The gypsy moth *Lymantria dispar* (Lepidoptera, Erebidae) potentially feeds on more than 600 plant species of 98 families (ILYINYKH et al., 2011), including approximately half of all tree species native to Europe (MONTGOMERY and WALLNER, 1988). However, the larvae exhibit considerable host preferences and optimal conditions for development and reproduction are only given on preferential host plants (WELLENSTEIN and SCHWENKE, 1978). In Central Europe, *Quercus robur*, *Quercus cerris* and *Quercus petraea* are the primary hosts (ALALOUNI et al., 2013). However, *L. dispar* shows very high genetic variation (WU et al., 2019) and different host plants are preferred in other parts of the vast range of distribution (McMANUS and CSÓKA, 2007).

The gypsy moth has a univoltine generation cycle and hibernates in the egg stage (WELLENSTEIN and SCHWENKE, 1978). In Austria, larval hatching usually starts in April (JAHN and SINREICH, 1957; SCHOPF and HOCH, 1997; KALBACHER, 2008) and the larvae develop through 5-6 (males) and 6-7 (females) instars, respectively, within 6-12 weeks (WELLENSTEIN and SCHWENKE, 1978). Ballooning – the dispersal supported by wind – of newly hatched larvae is primarily responsible for the distribution of gypsy moth populations (BARBOSA and CAPINERA, 1978). The first three instars (L1-L3) feed gregariously during the daytime, older larvae feed solitary at night and hide in bark fissures, the ground litter, or other protected resting places on the tree trunk or near the tree base during daytime (WELLENSTEIN and SCHWENKE, 1978). In Central Europe, pupation usually starts in June (HOCH, 1995; KALBACHER, 2008) and extends up to the end of August. Pupae are spun to branches, twigs, or the trunk of host trees; pupal development takes 10-23 days (WELLENSTEIN and SCHWENKE, 1978).

The flightless females of the European gypsy moth (*Lymantria dispar dispar*) (WU et al., 2019) deposit their eggs primarily on tree trunks or the underside of branches in late summer. In general, a single egg mass is oviposited by one female, typically contains 250-700 eggs, and is covered with brown abdominal hair (WELLENSTEIN and SCHWENKE, 1978; MONTGOMERY and WALLNER, 1988).

1.3 Distribution of *Lymantria dispar*, ecological and economic impact

Lymantria dispar is native to wide parts of the temperate, palearctic zones of Eurasia and northern Africa and was introduced to North America in 1869 (KEENA et al., 2008), where it has spread over large parts of the north-eastern U.S. and south-eastern Canada (FUESTER et al., 2014). Today, the gypsy moth is considered as the most important defoliator of deciduous hardwoods in the North American area of infestation (MONTGOMERY and WALLNER, 1988). In 1990, more than 15 million hectares were defoliated (LIEBHOLD et al., 1993). Currently, gypsy moth invasion via seaports threatens numerous countries, such as Uruguay, Brazil, South Africa, Australia, and New Zealand, which all – at least in some regions – offer suitable climatic conditions for successful insect development (PAINI et al., 2018).

Large gypsy moth populations may totally defoliate deciduous forests in early summer (WELLENSTEIN and SCHWENKE, 1978) and particularly pure stands with preferred food plants are endangered (MUZIKA and GOTTSCHALK, 1995). While a single year of defoliation

is usually not sufficient to kill deciduous trees (ALALOUNI et al., 2013), it impacts tree growth and seed production negatively. Several successive years of defoliation or synergistic effects with fungal (e.g., *Oidium alphitoides*) (WELLENSTEIN and SCHWENKE, 1978) or other insect pests (e.g., *Agrilus biguttatus*), as well as abiotic stresses may foster mortality. This may result in ecological effects (e.g., changes in tree composition), strong economic impacts (ALALOUNI et al., 2013), up to mortality of large forest stands (WELLENSTEIN and SCHWENKE, 1978).

In Europe, the gypsy moth is one of the main oak defoliators (HLÁSNY et al., 2016). Damage increases from west to east, and from north to south (WULF and GRASER, 1996; HLÁSNY et al., 2016). Particularly the warm and arid continental climate of the Balkan Peninsula offers optimal conditions for the development and suffered from population outbreaks that affected up to 1.5 million hectares in the past (WELLENSTEIN and SCHWENKE, 1978). In 1957, approximately 70 % of all hardwood forests in the countries of the former Yugoslavia were defoliated (MONTGOMERY and WALLNER, 1988). Outbreaks in Central Europe are less frequent and restricted to much smaller areas (WELLENSTEIN and SCHWENKE, 1978; WULF and GRASER, 1996). In Austria, gypsy moth gradations were documented in the 1930s, 1950s (JAHN and SINREICH, 1957), 1970s (FUESTER et al., 1983), 1990s (KREHAN, 1993), and the early 2000s (KALBACHER, 2008), with a maximum of approximately 2,500 hectares of infested area and 475 hectares of defoliation (KREHAN, 1993). Since 2002, the damage from the gypsy moth in Austrian forests is documented by the Austrian Research Centre for Forests (BFW) **(Fig. 1)**. While the damage was negligible from 2006, a heavy gradation occurred in 2018 and 2019 with more than 4,000 hectares of infested area (BFW, s.a.). ZÚBRIK et al. (2021) reviewed all outbreaks in Slovakia since 1945, which showed high correlation to the dynamics in Austria. No control measures were conducted since the early 1960s in Austria; however, in neighbouring countries biological and chemical insecticides are still widely used (KREHAN, 1993; HOCH et al., 2001; ZÚBRIK et al., 2021).

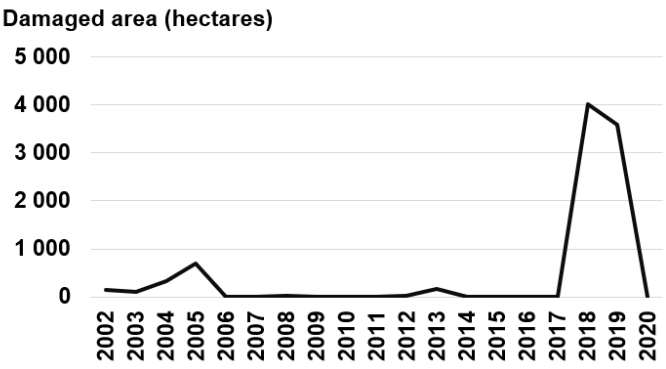

Fig.1: Area of Austrian forests damaged by the gypsy moth according to Documentation of Forest Damaging Factors (BFW, s.a.).

1.4 Population dynamics of *Lymantria dispar*

As with many other foliage feeding forest insects, gypsy moth populations follow periodic gradation cycles. Outside of the centres of distribution, outbreaks typically occur repeatedly in certain foci, i.e., forests that offer particularly favourable conditions for the gypsy moth development. However, large scale outbreaks seem to be synchronized across large regions, such as wide parts of Europe (JOHNSON et al., 2005; ALALOUNI et al., 2013; HLÁSNY et al., 2016).

Population cycles comprise latency periods with low population density, usually extending for 7-10 years in Central Europe (ALALOUNI et al., 2013). Successive years with warm, dry, and sunny weather conditions in spring are assumed to be the major trigger for the transition into the prograddation period (WELLENSTEIN and SCHWENKE, 1978; HOCH et al., 2001). During prograddation, fecundity and the proportion of females in the population increase, resulting in exceeding of the outbreak threshold and an epidemic population eruption within short time

(SCHÖNHERR, 1989). The peak population density is reached in the culmination period, before it decreases rapidly in the retrogradation period (ALALOUNI et al., 2013).
Many studies discussed the complex abiotic and biotic factors potentially impacting population dynamics of *L. dispar*, and most of them highlighted the density-dependent mortality by natural enemies as the most important regulating factor (LIEBHOLD et al., 2000; ALALOUNI et al., 2013).

1.4.1 Natural enemies of *L. dispar* and their role in population dynamics

Each group of natural enemies of *L. dispar* – parasitoids, predators and pathogens – may show significant regulative capacity in distinct periods of the gypsy moth population cycle (HOCH et al., 2001). This contributes to the fact that damage by the gypsy moth is generally much lower in Europe than in newly invaded areas, where the natural enemy complex is less divers (ŽIKIĆ et al., 2017). However, since 1906, numerous parasitoids and predators (CLAUSEN, 1978), as well as some pathogens were introduced and released – mainly from Europe – to the United States. Today, 13 parasitoid, three predator and two pathogen species have established in North America, most of them already before 1920 (FUESTER et al., 2014).

1.5 Parasitoids of *Lymantria dispar*

The term parasitoid refers to arthropods characterized by the feeding behaviour of their larvae, which feed exclusively on the body of one single arthropod host, and finally kill their host towards the end of their larval development (GODFRAY, 1994). Parasitoid lifestyle is known within seven insect orders. Approximately 80 % of all parasitoid species are members of Hymenoptera and the majority of non-hymenopteran parasitoids belongs to the order Diptera (QUICKE, 2015). More than 100 hymenopteran and dipteran species are described as parasitoids of eggs, larvae, or pupae of *L. dispar* in Europe (ŽIKIĆ et al., 2017), although particularly older listings include multiple references of synonyms and dubious records. FUESTER and RAMASESHIAH (1989) state a number of 45 species in Europe. Twenty species are found consistently in European field populations (McMANUS and CSÓKA, 2007). The highest overall parasitization rates are usually observed in post-culmination periods, during retrogradation and the early latency period after the population collapse. However, the relative importance of different parasitoids varies with the host population density and distinct families or species show their highest effects in different population phases of the gypsy moth, according to their biological features (HOCH et al., 2001; ALALOUNI et al., 2013).

1.5.1 Impact of parasitoids on the population dynamics of *L. dispar*

Egg parasitization rates of 64-96 % were observed in Turkey (AVCI, 2009) and typically range from 20-40 % in parts of North America (BROWN and CAMERON, 1982). In Central Europe, egg parasitism is considered to have low significance on the gypsy moth population density, with egg parasitization rates of 0-10 %, mainly caused by the eupelmid wasp *Anastatus disparis* Ruschka and rarely by the encyrtid wasp *Ooencyrtus kuvanae* Howard (FUESTER et al., 1983; BATHON, 1993; ZÚBRIK and NOVOTNÝ, 1997; ALALOUNI et al., 2013). Reports of egg parasitism in Austria are rare. FUESTER et al. (1983) observed *A. disparis* and a single specimen of *O. kuvanae* in the 1970s, while HOCH et al. (2001) did not detect any egg parasitoid in 60 egg masses inspected in the 1990s.

Gypsy moth pupae are attacked by few parasitoid species, most species are larval or larval-pupal parasitoids (ŽIKIĆ et al., 2017), which are important regulators of gypsy moth population density in latency periods and are involved in the collapse of population outbreaks. However, parasitoid populations cannot keep up in the progradation period, when gypsy moth

populations increase by several orders of magnitude within a single generation (WELLENSTEIN and SCHWENKE, 1978; ELKINTON and LIEBHOLD, 1990). The composition of the parasitoid complex varies between the native and artificial area of the gypsy moth distribution. While a low number of polyphagous, generalist parasitoids are predominant in North America, species diversity is higher in Europe (McMANUS and CSÓKA, 2007) and more specialized, oligophagous parasitoids predominate (HOCH et al., 2001).

Hymenopteran larval parasitoids – particularly Braconidae and Ichneumonidae – are important regulators at low and increasing host population densities, due to their biological and behavioural characteristics (ALALOUNI et al., 2014). They are often oligo- or multivoltine, have a high host searching capacity, and are not specific to *L. dispar* (ALALOUNI et al., 2013). However, some braconid species are essentially dependent on suitable alternative hosts for hibernating, which might limit their reproductive capacity at elevated gypsy moth population densities (HOCH et al., 2001).

The vast majority of dipteran parasitoids of *L. dispar* are tachinids (WELLENSTEIN and SCHWENKE, 1978; ŽIKIĆ et al., 2017). The delayed density-dependent numerical response of univoltine tachinids results in their very high abundance during periods of high host density, which can reduce the gypsy moth population size during outbreaks significantly (MONTGOMERY and WALLNER, 1988; ALALOUNI et al., 2013). Peak parasitism by tachinids usually occurs in the early post-culmination period (HOCH et al., 2001). After the collapse of gypsy moth outbreaks, also the tachinid populations collapse (MONTGOMERY and WALLNER, 1988). At this stage they may also be outperformed by parasitoids of younger host stages, such as braconids (HOCH et al., 2001). Braconids generally have a competitive advantage over tachinids, since they attack younger hosts and develop faster (MAIER, 1990).

Besides host death caused by development of their larvae (reproductive mortality), parasitoids can also negatively impact their hosts in several other ways, which are often underrated. Host feeding by female wasps, as well as mechanical damage (mutilation), or the injection of venoms or symbiotic viruses (pseudoparasitism) during host probing were shown to cause higher host mortality than the reproductive mortality in some cases. Sublethal effects are also possible. For example, immune defence costs can result in reduced fecundity of host individuals that survived a parasitoid attack (ABRAM et al., 2019). Parasitoids can act as vectors and transmit entomopathogens (REARDON and PODGWAITE, 1976), they can increase the virulence of pathogens in co-infested hosts (GODWIN and SHIELDS, 1984), and parasitoid invasion often results in host death without successful parasitoid emergence (GODWIN and ODELL, 1984), particularly in less suitable hosts (HERTING, 1960).

1.5.2 *Anastatus disparis* Ruschka (Hym., Eupelmidae)

Anastatus disparis is native to wide parts of Eurasia and North Africa (SULLIVAN et al., 1977; PEMBERTON et al., 1993). The species is known as egg parasitoid of several noxious lepidopteran species, including *L. dispar* (LIU et al., 2017a), but also representatives of Hemiptera. Further, *A. disparis* is a rare hyperparasitoid of the primary gypsy moth parasitoids *Cotesia melanoscela* (Ratzeburg) and *Oencyrtus* sp. (KURIR, 1944; GRIFFITHS, 1976). There are considerable differences in biology within the native and artificial area of its distribution. In Europe, the generation cycle of *A. disparis* is usually univoltine and very well synchronized with *L. dispar* (KURIR, 1944), although 2-3 generations per year were observed in Italy (CAMERINI, 2009). In North America, a second generation is occasionally observed (GRIFFITHS, 1976), and 3-4 generations per year are reported from China (LIU et al., 2017a).

The preference of *A. disparis* for freshly laid gypsy moth eggs for oviposition is described unanimously in the literature, while observations on the suitability of older gypsy moth eggs as hosts diverge. North American sources state that eggs are suitable during the whole embryonic development of *L. dispar* (GRIFFITHS, 1976), which lasts approximately 3-4 weeks (WELLENSTEIN and SCHWENKE, 1978), but the sex ratio of progenies becomes strongly male-biased in older eggs (GRIFFITHS, 1976). Despite that also dead gypsy moth eggs are described as suitable for the reproduction of *A. disparis* (CLAUSEN, 1978). In contrast, the Croatian population investigated by KURIR (1944) accepted host eggs only for the first three days after oviposition by *L. dispar*. The size of host eggs impacts the sex ratio of *A. disparis*, and it is speculated that *L. dispar* is not an optimal host due to its relatively low egg size, resulting in male-biased sex ratios (LIU et al., 2017a).

Anastatus disparis is a solitary parasitoid and although several females may oviposit the same egg, only one wasp develops per host egg (KURIR, 1944). The larval development of *A. disparis* takes 2-3 weeks, throughout which the entire content of the host egg is consumed. Subsequently, *A. disparis* hibernates in the host egg as mature larva (SULLIVAN et al., 1977), pupates in spring (GRIFFITHS, 1976), and emerges as adult wasp from the host egg **(Fig. 16C)** at the time of oviposition of *L. dispar*, from mid-June to mid-August, in a protandrous fashion (PARKER, 1933). Pupal exuviae and faeces remain in the host egg chorion and allow the differentiation between parasitized and non-parasitized host eggs after hatching (KURIR, 1944). Adults show considerable sexual dimorphism in morphology **(Fig. 16A-B)**, behaviour, and physiology (LIU et al., 2020a). Males are markedly smaller, can fly for short distances (KURIR, 1944; LIU et al., 2020a), and show a pronounced fighting behaviour with increasing group size, which often leads to the death of individuals (LIU et al., 2017b). Females are flightless but can jump very quickly and are often carried several hundred meters by wind in the field (CLAUSEN, 1978). Their ovipositor is short, consequently only the upper layer of eggs in an egg mass is parasitized (ZÚBRIK and NOVOTNÝ, 1997). Reports on the fecundity of *A. disparis* are strongly varying. While KURIR (1944) describes “the number of eggs oviposited by a fertilized female” as very low, ranging from 2-13 eggs, Chinese sources report similar egg numbers as the daily fecundity maintained for an average of 38 days (LIU et al., 2020b) and resulting in a lifelong fecundity of approximately 420 eggs per mated female (LIU et al., 2017a). Host feeding of adult females on gypsy moth eggs (PARKER, 1933) increases the fecundity of females (CLAUSEN, 1978).

In its native area of distribution *A. disparis* occurs only locally and periodically (BURGESS and CROSSMAN, 1929; WELLENSTEIN and SCHWENKE, 1978; LIU et al., 2017a). It is assumed that this is mainly caused by the females’ inability to fly (KURIR, 1944; SULLIVAN et al., 1977). Between 1906 and 1932, more than 74 million specimens of *A. disparis* were released in North America (GRIFFITHS, 1976) and egg parasitization rates and the impact on gypsy moth population dynamics are greater since it became established than in Europe (BESS, 1961; SULLIVAN et al., 1977).

1.5.3 *Glyptapanteles liparidis* (Bouché) (Hym., Braconidae)

Glyptapanteles liparidis is a gregarious, multivoltine braconid wasp, distributed all over Eurasia (PEMBERTON et al., 1993; SCHOPF and HOCH, 1997). The species hibernates in the larval stage inside lepidopteran host larvae (SCHOPF, 2007) and accordingly requires alternative hosts besides *L. dispar*. Consequently, the abundance of *G. liparidis* depends on the availability of suitable alternative hosts. *Dendrolimus pini* L. (Lep., Lasiocampidae) is reported as the most prominent hibernating host in Europe (BURGESS and CROSSMAN, 1929; GRIFFITHS, 1976; RAFFA, 1977; ČAPEK, 1988) and particularly high parasitization rates of *L. dispar* are reported from oak forest stands mixed with *Pinus sylvestris* (FUESTER et al.,

1983; ČAPEK, 1988). *Euproctis chrysorrhoea* L. (Lep., Erebidae) is also described as an alternative host (ČAPEK, 1988; MARSCHNIG, 2013; FROMM, 2014).

Glyptapanteles liparidis has 3-4 generations per year (ČAPEK, 1988), two of them develop in *L. dispar*. Adults **(Fig. 23B)** of the hibernating generation emerge in spring and attack young gypsy moth larvae, resulting in a summer generation of adult wasps. Females of the summer generation attack medium and large *L. dispar* larvae and their progenies attack alternative host larvae (BURGESS and CROSSMAN, 1929). The first three instars of *L. dispar* are considered as preferred host stages (SCHOPF and HOCH, 1997); however, almost all instars are attacked and the number of emerging larvae per host increases with host size. While 2-3 larvae develop from small hosts, 80-100 larvae can develop in mature gypsy moth larvae (GRIFFITHS, 1976). On average, 10-30 eggs are injected into a single host and females have 150-170 mature eggs in their ovaries at the same time (SCHOPF, 2007), resulting in up to more than 600 eggs throughout their lifetime (WIESER, 2019).

As members of the subfamily Microgastrinae (QUICKE, 2015), *G. liparidis* (TILLINGER et al., 2004) – as well as *Glyptapanteles porthetriae* (NUSSBAUMER et al., 2002), *Cotesia melanoscela*, and some ichneumonid wasps including *Hyposoter* sp. (STOLTZ et al., 1986) – have evolved effective strategies to interfere with the host metabolism, resulting in suppressed immune response and disrupted endocrine balance in the host larva. This is mediated by symbiotic polydnaviruses (PDVs) and venoms, which are secreted into the host haemolymph during oviposition, as well as by teratocytes, specialized cells with immunological, antimicrobial, and hormonal functions (SCHAFELLNER et al., 2007), which develop from the egg serosa parallel to the larval development in the host (QUICKE, 2015).

Glyptapanteles liparidis is among the dominant parasitoids of *L. dispar* in eastern Austria (SCHOPF and HOCH, 1997), was observed highly consistent in Austria in the past (FUESTER et al., 1983; EICHHORN, 1996; HOCH et al., 2001; KALBACHER, 2008), and is considered as one of the most efficient parasitoids of the gypsy moth in Europe (BURGESS and CROSSMAN, 1929) and Asia. Both the spring generation of the parasitic wasps in young gypsy moth larvae and the summer generation of the wasps in older larvae frequently cause parasitization rates of more than 20 % (FUESTER and RAMASESHIAH, 1989). Repeated intensive efforts to establish the species in North America failed (GRIFFITHS, 1976), probably due to the absence of suitable alternative hosts (RAFFA, 1977).

Glyptapanteles liparidis is particularly abundant in latency and progradation periods (ALALOUNI et al., 2013) and is able to immediately react to locally augmented host densities (HOCH et al., 2001). The high reproductive capacity – with a bivoltine development on *L. dispar* and a high number of progenies per host individual – and its highly sensitive host searching ability may regulate potential reproductive foci of the gypsy moth, and consequently prevent the transition from the latency to the progradation period. As gradations are probably often nipped in the bud in this way, this influence is certainly underestimated (SCHOPF and HOCH, 1997). At elevated host and parasitoid population densities, the whitish cocoons **(Fig. 23A)** that form unregular clusters on tree trunks or branches are very conspicuous (ČAPEK, 1988).

1.5.4 *Glyptapanteles porthetriae* (Muesebeck) (Hym., Braconidae)

Glyptapanteles porthetriae is a strictly solitary (SHAW and SKELTON, 2008) and multivoltine parasitoid wasp (BURGESS and CROSSMAN, 1929). The first larval stage of *G. porthetriae* (NUSSBAUMER and SCHOPF, 2000) hibernates inside larvae of *Euproctis chrysorrhoea* and other unknown lepidopteran species (ČAPEK, 1988). Usually, only females of the spring generation – that emerged from the overwintering hosts – attack larvae of *L. dispar,* while the

females that emerged from gypsy moth larvae attack other host species (BURGESS and CROSSMAN, 1929; FUESTER et al., 1983; ČAPEK, 1988). SHAW and SKELTON (2008) speculate that *G. porthetriae* could eventually hibernate in egg larvae of *L. dispar*, thus exhibiting a univoltine life cycle. However, in experiments by SCHAFELLNER (personal communication), neither *G. porthetriae* nor *G. liparidis* wasps were able to parasitize egg larvae of *L. dispar* successfully.

The first two instars of *L. dispar* are preferred as hosts (GRIFFITHS, 1976; MARKTL et al., 2002), while parasitization success and development in (late) third instars and older larvae are significantly impaired. Host larvae are mostly killed in the third or fourth instar, usually in the next instar following the attacked one. The last host instar is significantly prolonged. In L5 and L6 hosts, parasitoid larvae fail to emerge from the host, even after successful endoparasitic development (NUSSBAUMER and SCHOPF, 2000). The endoparasitic development is very similar to that of *G. liparidis*, however, *G. porthetriae* develops faster (MARKTL et al., 2002). After the emergence from the host, the parasitoid larva spins a whitish cocoon **(Fig. 21A-B)** that is loosely attached to the host larva. The host remains alive for several days and eventually protects the cocoon from hyperparasitoids and predators (ČAPEK, 1988).

Glyptapanteles porthetriae is widely distributed in Europe, but only occasionally abundant (GRIFFITHS, 1976), in many cases of minor importance (BURGESS and CROSSMAN, 1929), and parasitization rates rarely exceed 10 % (ČAPEK, 1988). Usually, *G. porthetriae* is most abundant during latency and progradation periods (ALALOUNI et al., 2013) and shows immediate reaction to augmented host densities (GRIFFITHS, 1976). However, *G. porthetriae* was also observed in high abundance during gradations and its relative importance compared to *G. liparidis* increases with the host density (MAKSIMOVIĆ and SIVČEC, 1984; ČAPEK, 1988; ALALOUNI et al., 2013). Since the host mostly dies already in the third or fourth instar, the impact of *G. porthetriae* on leaf damage is markedly higher as for parasitoids killing the host in later stages because the feeding activity of gypsy moth larvae dramatically increases during the final instars (NUSSBAUMER and SCHOPF, 2000).

1.5.5 *Cotesia melanoscela* (Ratzeburg) (Hym., Braconidae)

Cotesia melanoscela is a strictly solitary (SHAW and SKELTON, 2008), oligophagous, bivoltine, and monoxenous parasitoid wasp, i.e., it does not depend on alternative hosts (MAIER, 1990). The main hosts in Europe are *L. dispar* and the satin moth, *Leucoma salicis* L. (Lep., Erebidae) (CLAUSEN, 1978). Although *C. melanoscela* may hibernate inside overwintering host larvae, such as *Euproctis chrysorrhoea* (ČAPEK, 1988), usually the fully developed diapausing larvae overwinter inside their yellowish cocoons (BURGESS and CROSSMAN, 1929). Consequently, cocoons of the hibernating generation are highly exposed to attacks by hyperparasitoids (GRIFFITHS, 1976) and predators (WESELOH, 1983). More than 35 species of Chalcididae and Ichneumonidae are known as hyperparasitoids of *C. melanoscela* (ČAPEK, 1988). Overwintering mortality often exceeds 95 % in North America (WESELOH, 1983) and reaches similar values in Europe (GRIFFITHS, 1976).

Adult wasps emerge from the hibernating cocoons at the peak time of *L. dispar* egg hatching (CLAUSEN, 1978) and mainly parasitize the first two gypsy moth instars, resulting in the summer generation of wasps. Females of the summer generation usually parasitize L3 and L4 host larvae, resulting in the next hibernating generation (BURGESS and CROSSMAN, 1929). Hibernating cocoons of *C. melanoscela* are consequently mainly found on the resting places of older gypsy moth larvae, while the cocoons from which wasps of the summer generation emerge are mainly found in the upper parts of trees (WESELOH, 1983). Females can lay from 500 to 1,000 eggs and attack 15 host larvae per day on average (GRIFFITHS, 1976).

Cotesia melanoscela is widely distributed throughout Europe, North Africa, and Asia (BURGESS and CROSSMAN, 1929; PEMBERTON et al., 1993). After its introduction in 1912 (BLACKBURN and HAJEK, 2018), it quickly became established and spread across North America. While the effectiveness is variable in Europe (GRIFFITHS, 1976) and Asia (FUESTER and RAMASHESHIAH, 1989), and *C. melanoscela* is not a parasitoid of primary importance in its native area of distribution (BURGESS and CROSSMAN, 1929), the species ranks among the most important gypsy moth parasitoids in North America (GRIFFITHS, 1976). The highest significance is observed in periods of low host density, and *C. melanoscela* is usually not able to respond effectively to increased host densities, as its high reproductive capacity is compensated for by the high rates of hyperparasitization (ČAPEK, 1988). However, high parasitization rates by *C. melanoscela* were also observed during gypsy moth outbreaks (ALALOUNI et al., 2013). Parasitization rates typically reach up to 50 % in North America and 25 % in Europe (FUESTER and RAMASHESHIAH, 1989).

1.5.6 *Phobocampe* spp. (Hym., Ichneumonidae)

Several species of the ichneumonid genus *Phobocampe* are known as solitary, univoltine, and probably oligophagous (MUESEBECK and PARKER, 1933; ŽIKIĆ et al., 2017) parasitoids of the gypsy moth, including *P. unicincta* (Gravenhorst), *P. lymantriae* Gupta, *P. disparis* (Viereck), and *P. pulchella* Thomson (HOCH, 1995; NOVOTNÝ et al., 1996; ALALOUNI et al., 2013). *Phobocampe* females attack young gypsy moth larvae with preference for the first instar and may oviposit more than 50 eggs per day and 1,000 eggs during their 5-8 weeks of adult lifetime. The emergence from the host typically takes place during the fourth host instar. The highly distinctive ovoidal cocoons are loosely spun to the host but fall to the ground soon. In most cases, the mature wasps overwinter inside the cocoon at the soil surface and adult eclosion starts at the time of the gypsy moth egg hatching (MUESEBECK and PARKER, 1933). However, in rare cases, adult eclosion is already observed in the year of the endoparasitic development (HOCH, 1995).

Phobocampe spp. are neither considered as gypsy moth parasitoids of major importance in Europe (MUESEBECK and PARKER, 1933; ALALOUNI et al., 2013) nor in North America (BLACKBURN and HAJEK, 2018). However, with parasitization rates of 10 to 25 %, which are frequently observed in sparse to moderately dense populations during post-culmination periods, *Phobocampe* species are the most abundant ichneumonid parasites of *L. dispar* in Austria (FUESTER et al., 1983; HOCH et al., 2001).

1.5.7 *Hyposoter tricoloripes* (Viereck) (Hym., Ichneumonidae)

Hyposoter tricoloripes is a solitary (FUSCO, 1981) and probably oligophagous parasitoid. Knowledge on the host specificity is poor, but it is assumed that an alternative host is required. Only one generation per year develops on the gypsy moth; however, the species shows no diapause and adults emerge in summer (FUESTER et al., 1983). The cocoons are highly distinctive and usually concealed by the skin of the host on their upside **(Fig. 25)** (HOWARD and FISKE, 1911). *Hyposoter tricoloripes* is a parasitoid of small larvae, first instar larvae are preferred in laboratory experiments (FUSCO, 1981). In the field the host is usually killed during the late third (FUESTER et al., 1981) or fourth instar (FUESTER et al., 1983).

Hyposoter tricoloripes is widely distributed in Europe (GUPTA, 1983); however, its abundance is very inconsistently and locally scattered. In general, it is considered as a rare parasitoid of *L. dispar*, although it can be an effective parasitoid at certain locations and years. Parasitization rates around 30 % in Austria (FUESTER et al., 1983) and up to 40 % in Slovakia (HOCH et

al., 2001) were observed in exceptional cases. *Hyposoter tricoloripes* is most effective at low host densities (FUSCO, 1981).

1.5.8 *Parasetigena silvestris* (Robineau-Desvoidy) (Dip., Tachinidae)

Parasetigena silvestris is a univoltine and monoxenous parasitoid, with a generation cycle strongly adapted to *L. dispar* (MAIER, 1990). It is a highly consistent and abundant parasitoid during outbreaks of its main hosts, the nun moth, *Lymantria monacha*, and *L. dispar* (TSCHORSNIG and HERTING, 1994), and rarely also emerges from *Euproctis chrysorrhoea* (CLAUSEN, 1956).

Parasetigena silvestris hibernates within a puparium in the soil, and adult flies emerge from May on. Females begin to oviposit approximately two weeks after mating and attach their white, macrotype eggs externally on the host cuticle, where they are visible to the naked eye **(Fig. 5)** (HERTING, 1960). All instars of *L. dispar* are accepted for oviposition (MAIER, 1990), although parasitization of L1-L3 hosts is rarely successful (HERTING, 1960). Movement of host larvae is considered as the main stimulus for oviposition, consequently oviposition by *P. silvestris* reaches its daily peaks at dawn and sunset when gypsy moth larvae migrate between their resting and feeding sites (GOULD et al., 1992). Additionally, phototaxic responses play a role in the stimulation of oviposition and probably also chemical signals are involved, since *P. silvestris* shows good host location abilities also at low host densities (ODELL and GODWIN, 1979).

On average, a female lays 10-20 eggs per day and around 115-200 eggs during its lifespan of approximately one month (HERTING, 1960). Usually, each female only places one egg per host, but multiple ovipositions by several females occur frequently and it is unclear if females can discriminate parasitized from unparasitized hosts. However, even in the case of multiple ovipositions, mostly only one tachinid fly develops successfully (MAIER, 1990; GOULD et al., 1992). Eggs are fertilized by females immediately prior to oviposition and depending on the temperature, it takes 3-8 days until the tachinid maggots hatch and bore into the host haemocoel directly from the egg. In many cases, the eggs get stripped off with the exuviae of moulting host larvae (HERTING, 1960). It is assumed that this way 20-50 % of *P. silvestris* eggs are lost prior to hatching (PRELL, 1915; MAIER, 1990).

Development of the maggot within the host takes 17-25 days. The first two instars develop slowly, subsist mainly from haemolymph, and hardly harm the host. During the parasitoids final instar, inner host organs disintegrate due to extraintestinal secretions of the maggot. The host is killed and quickly consumed by the parasitoid, resulting in rapid maggot growth (HERTING, 1960). The tachinid maggot mostly emerges from the fully mature host larva, prior to its pupation (SABROSKY and REARDON, 1976). After egression from the host, the maggot flops to the ground, digs into the soil and pupates (HERTING, 1960).

Parasetigena silvestris is widely spread in Eurasia (PEMBERTON et al., 1993), as well as in North America, where it has established after 1927 (SABROSKY and REARDON, 1976). The tachinid species is the dominant parasitoid of *L. dispar* in large parts of Eurasia (LEE and PEMBERTON, 2019), including Austria (FUESTER et al., 1983; EICHHORN, 1996; HOCH et al., 2001; KALBACHER, 2008). It is very consistently found at all gypsy moth population densities, particularly during culmination and retrogradation periods, in which it can reach very high parasitization rates (ALALOUNI et al., 2013). In Austria, 40 % mortality in L5 and L6 larvae was observed by HOCH et al. (2001).

1.5.9 *Blepharipa pratensis* (Meigen) (Dip., Tachinidae)

Similar to *P. silvestris*, *Blepharipa pratensis* is also a monoxenous, univoltine, solitary, and specialized tachinid species. It is a larval-pupal parasitoid primarily of *L. dispar* and *Dendrolimus pini*, rarely of other lepidopteran hosts (TSCHORSNIG and HERTING, 1994), and frequently found in deciduous and pine forests of the warmer regions of Europe (HERTING, 1960).

Blepharipa pratensis overwinters within puparia in the soil and adult flies emerge 1-2 weeks prior to the peak of the gypsy moth egg hatching (CLAUSEN, 1956). The peak of adult flight is reached usually from mid-May to mid-June (TSCHORSNIG and HERTING, 1994). Females of *B. pratensis* exhibit an indirect oviposition strategy, using tiny, so called microtype eggs (HERTING, 1960). Females of microoviparous tachinid species move from leaf to leaf during daytime, when gypsy moth larvae are usually not present at their feeding places, due to their diel periodicity. Tachinid females perceive exudates of damaged leaves (e.g., sugars), which induce an arresting behaviour and examinations of the leaf surface with their front tarsi. Subsequently, the perception of mechanical damages induces females to take up the pre-oviposition position, and chemical elicitors originating from the host larvae (e.g., silk strands or regurgitates) seem to elicit the actual oviposition on the edge of the leaf (ODELL and GODWIN, 1984). Females of *B. pratensis* deposit up to 3,200 to 5,000 eggs during their lifespan (SABROSKY and REARDON, 1976), which contain fully developed egg larvae (HERTING, 1960).

Eggs of *B. pratensis* are taken up by host larvae with food. The mandibles of young *L. dispar* instars crack the tough egg chorion; however, the elastic vitelline membrane usually pops out of the chorion. From the fourth host instar, the probability for tachinid maggots to survive this process is higher than to die (GODWIN and ODELL, 1981) and L4 hosts can also swallow whole eggs. After ingestion, *B. pratensis* maggots hatch inside the host gut, enter the haemocoel, and invade the intersegmental muscles within 4-20 hours (SHIELDS, 1976) to escape the host immune defence reactions (MAIER, 1990; GODFRAY, 1994). First-instar maggots enter an endogenous diapause and virtually show no growth until the host reaches the prepupal stage. Consequently, development and growth of first-instar maggots depends highly on the host development. During the prepupal host stage, the maggot moults and enters the host haemocoel as second-instar larva. The inner organs of the host disintegrate due to extraintestinal secretions, and the growth of the tachinid maggot increases significantly. The host is killed usually 3-4 days after its pupation and the mature third-instar maggot egresses the pupa around day seven after pupation (HERTING, 1960; SHIELDS, 1976).

Gypsy moth larvae consume a higher amount of foliage in their last instar than in all other instars combined and females have an additional and prolonged final instar compared to males (LEONARD, 1981; ANDRAE, 2013). The number of *B. pratensis* eggs on leaves increases as the season progresses (GODWIN and SHIELDS, 1984) and the proportion of maggots that survive the egg ingestion increases with the host instar. Consequently, *B. pratensis* is a particularly efficient parasitoid of mature (GODWIN and ODELL, 1981) and female gypsy moth larvae (SABROSKY and REARDON, 1976; FUESTER and TAYLOR, 1996). Since the host dies late after its complete larval development and is virtually not affected negatively until its prepupal stage, the amount of foliage consumed is hardly affected by *B. pratensis*. Accordingly, the attenuation of leaf damage is significantly less than with parasitoids which kill their host in earlier stages (SABROSKY and REARDON, 1976).

Blepharipa pratensis is among the dominant gypsy moth parasitoids in Eurasia and North America, where it has become established since the early 20th century. The parasitic fly often

acts as a significant factor in the population collapse after outbreaks (SABROSKY and REARDON, 1976). High parasitization rates are observed during prograding, culmination, and particularly retrogradation periods, and can reach up to 95 % (ALALOUNI et al., 2013). *Blepharipa pratensis* was recorded very consistently also in Austria with parasitization rates up to 35 % (EICHHORN, 1996); however, parasitization rates were lower than those caused by *P. silvestris* (FUESTER et al., 1983; HOCH et al., 2001; KALBACHER, 2008).

1.5.10 *Blepharipa schineri* (Mesnil) (Dip., Tachinidae)

Blepharipa schineri is closely related to *B. pratensis* and shows minor differences in its biology (MAIER, 1990). *Blepharipa schineri* is described as the Asian congener of *B. pratensis* and is the dominant *Blepharipa* species in the Far East, where parasitization rates up to 100 % were observed (FUESTER and RAMASHESHIAH, 1989; PEMBERTON et al., 1993; FUESTER and TAYLOR, 1996). It was not deliberately released in North America, due to concerns about competitive effects to *B. pratensis* and *P. silvestris* (McMANUS and CSÓKA, 2007). However, it was introduced unconsciously in low numbers but failed to establish (SABROSKY and REARDON, 1976). In Europe, the species occurs in common with *B. pratensis* but is much less abundant (HERTING, 1960; TSCHORSNIG and HERTING, 1994). In Germany (MAIER, 1990) and France (HÉRARD and CHEN, 1998), however, *B. schineri* was observed as the clearly dominant *Blepharipa* species. Since the puparia of the two species are indistinguishable, MAIER (1990) assumed that both species were confused in many studies, and *B. schineri* might be much more abundant than generally believed. In Austria, the study of HOCH et al. (2001) – with species identification based on adults dissected out of puparia – did not support this hypothesis.

1.6 Pathogens of *Lymantria dispar* and their impact on population dynamics

In a broad sense, pathogens are defined as parasitic microbial agents, causing infectious diseases of their hosts. Direct transgenerational transmission of entomopathogens from infected hosts to their offspring is referred to as vertical transmission and can be further divided to a transovarial and a transovum pathway. While transovarian infections term infections of the embryo and occur within the female's ovaria, transoval transmissions are restricted to the egg surface and occur after oviposition. Horizontal transmission refers to all direct infections between host individuals, except those from parents to the offspring. The horizontal pathway typically occurs within one host generation; however, transmission can also be transgenerational, via contaminated environmental surfaces (SOLTER and BECNEL, 2018).

Successful infections require the contact of a pathogen inoculum with host organs that allow an infection (HAJEK and SHAPIRO-ILAN, 2018). Most viruses, bacteria and microsporidia enter their hosts via the gastrointestinal tract, most fungi penetrate the cuticle (KAYA and VEGA, 2012). Most entomopathogens act in a density dependent fashion and cause the highest mortality rates at elevated host densities (HOCH et al., 2001). This correlates with higher releases of inocula (SHAPIRO-ILAN et al., 2012) and a higher chance for host-pathogen encounter, since pathogen transmission is a random process (HAJEK and SHAPIRO-ILAN, 2018). Furthermore, insect herbivore population outbreaks often result in reduced food availability and quality, which correlates with worse constitution of the host larvae (WELLENSTEIN and SCHWENKE, 1978). Additionally, starvation increases the susceptibility of insects to entomopathogens significantly (PAVLUSHIN et al., 2021).

1.6.1 Impact of pathogens on the population dynamics of *Lymantria dispar*

L. dispar acts as a host for a diverse complex of pathogens, including viruses (McMANUS and CSÓKA, 2007), fungi (HAJEK et al., 1997), microsporidia (McMANUS and SOLTER, 2003), and bacteria (NOVOTNÝ, 1989; DEMİR et al., 2012). In Central Europe, pathogens generally cause higher mortality in elevated gypsy moth populations than parasitoids (HOCH et al., 2001; ALALOUNI et al., 2013). Although the number of pathogen species infecting *L. dispar* is markedly lower than the number of parasitoid species, also pathogens are highly divers due to a large variety of pathogenic strains. These may strongly vary in their general pathogenicity to a distinct host and their degree of virulence (SHAPIRO-ILAN et al., 2012). For example, the virulence of different isolates of the *Lymantria dispar* multinucleocapsid nuclear polyhedrosis virus (*Ld*MNPV), a naturally occurring baculovirus, varies for at least one order of magnitude (AKHANAEV, et al., 2020).

1.6.2 *Lymantria dispar* multinucleocapsid nuclear polyhedrosis virus (Ld*M*NPV, NPV) (Baculoviridae)

Multinucleocapsid nuclear polyhedrosis viruses are a large group of viruses associated with a broad spectrum of insects. *M*NPVs are characterized by double-stranded circular DNA and biphasic infection cycles, initiated by oral uptake of polyhedrally shaped occlusion bodies (OBs) **(Fig. 40A)**, in which numerous virions are embedded (IKEDA et al., 2015). Ld*M*NPV is highly host specific to *L. dispar* (SOLTER and HAJEK, 2009) and will be referred to as NPV in this work from now on.

Horizontal transovum transmission to newly hatched larvae, which feed on externally OB-contaminated egg chorions was traditionally considered as the major pathway of initial NPV infections. Vertical transmission and sublethal infections were considered to be of minor importance in the past, due to the high virulence of NPV (ELKINTON and LIEBHOLD, 1990; YERGER and ROSSITER, 1996; MYERS and CORI, 2015); however, both processes – which are closely related and partially depend on each other – are proven for NPV and recently increased interest in research showed that they were underestimated in the past (PAVLUSHIN et al., 2019; AKHANAEV et al., 2020).

Horizontal transmission originates from viral occlusion bodies, the resting form of NPVs that can persist for several years outside their host. However, this presupposes protection from sunlight, due to their high sensitivity against UV-radiation (MURRAY et al., 1989). After oral uptake, OBs are dissolved by alkaline secretions in the midgut, resulting in the dissolution of the polyhedral matrix and the release of so-called OB-derived virions (ODVs). This induces a complex biphasic infection cycle, resulting in viral reproduction. Phase I is restricted to midgut cells and the production of budded virions (BVs). BVs released from the nucleus are responsible for cell-to-cell transmissions in an infected host. The second phase takes place in various host organs and involves the formation of BVs and ODVs. ODVs are imbedded in OB matrices, remain in the nuclei and do not contribute to further reproduction within the host individual (IKEDA et al., 2015). The formation of OBs is initiated about 48 hours post infection (SOLTER and HAJEK, 2009), however, viral replication follows an exponential growth. Consequently, the great majority of OBs is formed within the last few days prior to host death (YERGER and ROSSITER, 1996).

The duration until host death depends on host size, infection dosage, temperature (SOLTER and HAJEK, 2009), viral isolate (AKHANAEV et al., 2020), and host constitution (PAVLUSHIN et al., 2021). While host death in older larvae may take up to 25 days from infection, the extremely susceptible neonate larvae (PÁEZ et al., 2015) mostly die within a range of about

13 days. Consequently, a first wave of host death by NPV typically occurs about 1-2 weeks after hatching (YERGER and ROSSITER, 1996). NPV infections result in liquefaction of the host cadavers and release of OBs immediately after death (AKHANAEV et al., 2020). Disintegrated cadavers of larvae from initial infections are attached to leaves and serve as inoculum for feeding larvae. This typically results in a second peak of disease about 3-4 weeks later and a bimodal pattern of NPV mortality prevalence (WOODS and ELKINTON, 1987; YERGER and ROSSITER, 1996). Dying larvae are usually attached to twigs or bark in an inverted "V"-shape, before the disintegration of the cadaver begins **(Fig. 36)** (SOLTER and HAJEK, 2009).

NPV is distributed throughout the native and invasive range of distribution of *L. dispar* (SOLTER and HAJEK, 2009) and has an outstanding significance on its population dynamics. NPV is considered as the most important gypsy moth pathogen worldwide, which is essentially involved in the collapse of virtually all population outbreaks during retrogradation (DOANE, 1970; WELLENSTEIN and SCHWENKE, 1978; WEISER, 1998; ALALOUNI et al., 2013). In addition to very high mortality rates, sublethal infections correlate with reduced pupal weights, altered sex ratio, as well as impaired adult fecundity, and increased egg mortality (IL'INYKH et al., 2009; PÁEZ et al., 2015). NPV acts strongly density dependent, however, high gypsy moth populations are not always associated with NPV epizootics and high NPV prevalence may be also observed at low population densities (HOCH et al., 2001; SOLTER and HAJEK, 2009). Intensive attempts were made to establish NPV as a commercial bioinsecticide in the U.S. in the past decades. In spite of its high efficacy, it could not prevail for large-scale practical use until today. This is attributed to the cost- and labour-intensive production (SOLTER and HAJEK, 2009). However, due to the exceptional host specificity and environmental safety it is still used for small areas harbouring endangered lepidopteran species in the U.S. (COLEMAN et al., 2020).

1.6.3 *Entomophaga maimaiga* Humber, Shimazu & Soper (Entomophthoromycota, Entomopthoraceae)

The fungal order of Entomophthorales comprises a large group of obligate pathogens, infecting a wide host range of arthropods, while single species or strains are quite host specific (HAJEK, 1999). In case of *E. maimaiga*, intensive evaluations showed a physiological host range restricted to lepidopterans, particularly lymantriids, and an ecological host range largely restricted to *L. dispar*, i.e., infections of other species were observed rarely in the field (SOLTER and HAJEK, 2009; ZÚBRIK et al., 2016).

Two types of spores are involved in the infection cycle of *E. maimaiga*, sexual azygospores and asexual conidia **(Fig. 40B)**. The thick-walled azygospores remain dormant in the year of formation and form soil reservoirs where they can persist and remain germinable for more than a decade. Germination of azygospores requires a preceding cold period, a critical photoperiodic day length (HAJEK, 1999, HAJEK et al., 2018), and a critical level of soil moisture (REILLY et al., 2014). Azygospores irregularly germinate over a period of approximately six weeks in May and June, however, a portion of azygospores always remains dormant in the soil reservoir. Germination results in the active ejection of infective germ conidia, which are responsible for the initial transgenerational transmission via penetration of the cuticle of the host larvae. It is assumed that initial infections primarily occur in or on the soil (HAJEK, 1999). Infected larvae are typically killed within 4-7 days (MALAKAR et al., 1999).

Entomophaga maimaiga passes through 6-7 (MALAKAR et al., 1999), occasionally nine infection cycles within one generation of host larvae (HAJEK and SHAPIRO-ILAN, 2018). Secondary infections are initiated by airborne conidia, which are actively discharged from the

surface of host cadavers. The type of spores formed on a host cadaver depends on several factors. While cadavers of larvae infected by germ conidia exclusively produce conidia, cadavers of larvae infected by airborne conidia are capable to form conidia as well as azygospores (HAJEK, 1999). The formation of azygospores increases with host age, temperature, humidity, and the dosage of inoculum (HAJEK et al., 2018).

Although *E. maimaiga* can infect all larval stages of *L. dispar*, the highest mortality rates are typically observed in old larvae. This can be explained by the multiple infection cycles, resulting in an exponential increase of airborne conidia with the progressing season (HAJEK, 1997). This is enhanced by the ability of airborne conidia which do not encounter to a host cuticle to actively discharge secondary conidia. Secondary conidia can discharge tertiary conidia and those quaternary conidia again. Although it is not known if quaternary conidia are infective, the probability of successful infections by airborne conidia is markedly increased this way (HAJEK, 1999).

While most entomopathogens clearly act host density dependent (SHAPIRO-ILAN et al., 2012), results of studies on the density dependence of *E. maimaiga* diverge. However, the majority of studies report density independence (SOLTER and HAJEK, 2009; HAJEK et al., 2015) and the prevalence of *E. maimaiga* seems to be much more affected by weather conditions than by host density, since germination of both azygospores and conidia strongly depends on temperature and humidity of soil and air (HAJEK et al., 1999; REILLY et al., 2014).

Entomophaga maimaiga is native to East Asia and established in North America since 1989, where it has spread very fast (SOLTER and HAJEK, 2009). Today, *E. maimaiga* has displaced NPV as the dominant pathogen of *L. dispar* in wide parts of North America and often is the dominant factor in the collapse of population outbreaks. Furthermore, it is assumed that gradations are suppressed by preventing the transition from latency to progradation period (HAJEK et al., 2015). The high efficacy of the pathogen in North America inspired Bulgarian authorities to releases in Bulgaria (PILARSKA et al., 2006a), which were conducted between 1999 and 2014 (PILARSKA et al., 2020). In the past 10 years, *E. maimaiga* was spreading towards Central Europe along the Balkan Peninsula. In 2013, it was detected in Hungary and Slovakia, where it became established and widespread (ZÚBRIK et al., 2018) and in 2014 screenings for the entomopathogen were started in Austria, which resulted in no detection in the first five years of screening (PILARSKA et al., 2020). Finally, in 2019, *E. maimaiga* was observed at two gypsy moth outbreak sites in Lower Austria (Eggenburg and Ebergassing) with high prevalence (HOCH et al., 2019), as well as in the Czech Republic. Most detection sites in the Czech Republic (HOLUŠA et al., 2020) were in close vicinity to Eggenburg, located approximately 30-80 km in the north-eastern direction from the present study site.

The high impact of *E. maimaiga* on *L. dispar* population dynamics may impact the host reservoir for other natural enemies of the gypsy moth negatively. Additionally, the fast development and rapid host death make *E. maimaiga* a superior competitor in case of co-infections with other pathogens and parasitoids (ELKINTON et al., 2019). Negative correlations between the prevalence of *E. maimaiga* and parasitism rates were shown in North America (HAJEK et al., 2015) and are supported by first European results. It is assumed that tachinids – as parasitoids associated with late host instars – suffer particularly from the competition (GEORGIEV et al., 2013).

1.6.4 Microsporidia

Microsporidia are eukaryotic unicellular organisms, living as intracellular parasites mainly in insects (MADDOX et al., 1996; McMANUS and SOLTER, 2003). Unlike NPV and *E. maimaiga*, which require host death for pathogen transmission, microsporidia can also be transmitted by living hosts (GOERTZ and HOCH, 2008a), they cause chronic diseases rather than host mortality (HAJEK and SHAPIRO-ILAN, 2018), and show a broader host range (SOLTER et al., 2010).

Seven microsporidian species that belong to three genera are described as pathogens of *L. dispar* in Europe (McMANUS and SOLTER, 2003). They differ in their transmission pathways, primary sites of infection, and their virulence (PILARSKA et al., 2006b). Infections of the highly virulent *Vairimorpha* spp. mostly result in host death in the late larval or pupal stage, even with low-dose infections. Consequently, few infected individuals reach the adult stage and vertical transmission plays a minor role (MADDOX et al., 1996). Disintegrating host cadavers release environmentally resistant spores after host death (MADDOX et al., 1996) and serve as main inocula for *Vairimorpha* (GOERTZ and HOCH, 2008a).

Nosema spp. show moderate virulence. While transovarially infected individuals often suffer high mortality in early instars, usually only heavy horizontal infections result in host death. Faeces serve as main inocula for *Nosema,* but resting spores are also released from host cadavers and larval silk glands. Consequently, transmission of *Nosema* is more efficient than the transmission of *Vairimorpha* (MADDOX et al., 1996; GOERTZ and HOCH, 2008a).

Endoreticulatus schubergi Zwölfer is characterized by low virulence and predominantly chronic infections causing sublethal effects, including effects on mating efficiency and slower larval development. The latter increases the exposition to other abiotic and biotic mortality factors (MADDOX et al., 1996), including parasitism by *Glyptapanteles* spp. and *C. melanoscela* (McMANUS and SOLTER, 2003). Infections by *E. schubergi* are restricted to epithelial midgut cells and the transmission occurs via infected cells, which are separated from the gut tissue and excreted with faeces. Transgenerational transmission is mediated by surface contaminations of egg chorions. The high protein content of the eliminated midgut cells makes them attractive as food for newly hatched larvae (WEISER, 1998).

Infections of *L. dispar* by microsporidia are exclusively reported within its native area of distribution and the highest prevalence is typically observed during progradation periods. Although prevalence up to 90 % and mortality rates up to 70 % caused by *Nosema* sp. were observed in the field (McMANUS and SOLTER, 2003), such cases are rare and microsporidia usually occur on enzootic levels in the field. Since microsporidia rarely occur in high prevalence and cause dramatic epizootics, they often remain undetected. Nevertheless, their lethal and sublethal effects can contribute to the regulation of the gypsy moth population density (MADDOX et al., 1996; HOCH et al., 2009). During culmination and retrogradation, the prevalence of microsporidia typically decreases with increasing NPV prevalence (McMANUS and SOLTER, 2003).

1.6.5 Bacteria

Bacteria often appear to be opportunistic pathogens of insects (HAJEK and SHAPIRO-ILAN, 2018), i.e., their pathogenicity requires certain conditions, like impaired host immunity (KAYA and VEGA, 2012). Studies of bacteria as pathogens of *L. dispar* are rare (DEMİR et al., 2012). NOVOTNÝ (1989) considers bacteria to be an important mortality factor in Europe, particularly in young larvae. *Streptococcus faecalis* Andrewes & Horder is commonly involved in epizootics

in North America (DOANE, 1970). Although *Bacillus thuringiensis* (Berliner) can be isolated from *L. dispar* (DEMİR et al., 2012), it does not typically occur in lepidopteran defoliators of oak and does not cause epizootics in *L. dispar*. However, *B. thuringiensis* is used as bioinsecticide against the gypsy moth in Europe (WEISER, 1998) and North America (SOLTER and HAJEK, 2009).

1.7 Interactions between mortality agents

The tritrophic interactions between parasitoids, pathogens and their hosts can be complex and of several different natures (BROOKS, 1993). A minority of parasitoids is able to recognize the presence of other parasitoid species in a host individual and avoid such hosts for oviposition (GODFRAY, 1994). In case of multiparasitism – the invasion of a host individual by more than one parasitoid species – typically only one species can successfully develop on the host and reproduce (ELKINTON et al., 1992). Multiparasitism can also result in host death without successful emergence of any parasitoid (GODWIN and ODELL, 1984).
Similar dynamics are observed after co-infections by different entomopathogens and in most cases only one species will successfully reproduce. However, also mixed infections with reproduction of both species and synergistic effects are possible (MALAKAR et al., 1999; PILARSKA et al., 2006b).

Most interactions between parasitoids and pathogens are disadvantageous for the parasitoid but in many cases advantageous for the pathogen. The most common type of deleterious interactions is the premature death of co-infested hosts, prior to the completion of the development of one of the competitors. Other negative interactions include toxin production and direct infection of the parasitoid larvae by entomopathogens or reduced ovipositional attractivity of the infected hosts. While several parasitoids act as vectors for entomopathogens and parasitization often increases host susceptibility to pathogens, hosts infected by pathogens show reduced nutritional and physiological suitability for parasitoids in most cases. Negative impacts on parasitoids developing in pathogen-infected hosts range from premature death to sublethal effects, including reduced fecundity or longevity of adult parasitoids (BROOKS, 1993).

The competitive interactions within the host between various *L. dispar* mortality agents are still largely unknown (GOULD, 1990). For several interactions a clear tendency was shown. For example, braconid parasitoids usually outcompete tachinids (MAIER, 1990) or *E. maimaiga* has a competitive advantage over NPV (MALAKAR et al., 1999) and several parasitoid species (ELKINTON et al., 2019). In all cases, the shorter development time within the host is blamed for the superiority. However, the competitive advantage can shift from one species to another depending on the host stage attacked, as is the case with *G. liparidis* and *G. porthetriae* (MARKTL et al., 2002). In many cases, the attack sequence is the major decisive determinant, and the species that attacks the host first is more likely to complete its development. This is the case, for example, with the competition between the microsporidia *Nosema* sp. and *Vairimorpha* sp. (PILARSKA et al., 2006b), or the tachinid flies *B. pratensis* and *P. silvestris* (GODWIN and ODELL, 1984).

1.8 Evaluation of mortality factors in insect survival studies

Stage-specific determinations of apparent mortality rates – the proportion of individuals evidently killed by a mortality factor in a distinct sample – are widely used in studies examining the entire complex of natural enemies of *L. dispar*. They offer simple, practicable and easily comparable measures for the impact of mortality factors on population dynamics. However, these methods give biased results due to several reasons. In the field, usually not all individuals

of a population are present in the same stage of development at the same time. For example, some individuals die or already moult to the fourth instar before other individuals moult from the second to the third instar. Thus, there is no point in time to draw a sample out of the statistical population of all L3 larvae. Mortality factors that kill the host in a stage other than the attacked stage are also problematic and tend to be overestimated. Stage-specific mortality rates for distinct mortality agents cannot be subsumed because the number of potential hosts varies from stage to stage. Consequently, this method only allows estimates of the influence of a mortality factor on a certain host stage, while the impact on the entire generation development cannot be quantified (VAN DRIESCHE, 1983; GOULD et al., 1989). Furthermore, apparent mortality is influenced by other mortality agents acting in the same host stage. In case of multiparasitism or co-infestations only one species will be scored as the apparent mortality agent, although the host would have been killed also in the absence of the apparent mortality agent (ELKINTON et al., 1992).

Several methods were developed to overcome these biases and discussed, for example by GOULD et al. (1989). A promising approach is the use of marginal attack rates and the killing power of mortality agents. The marginal attack rate describes the theoretical proportion of individuals entering a particular stage that would be attacked and killed by a particular mortality agent if no other mortality agents would act simultaneously. The killing power (or key-value) *k* is a measure for the efficacy to kill hosts. The higher *k* the higher is the impact on the host population. *k* can be added to stage-specific values (comprising all mortality factors) or to factor-specific values as well, describing the impact of one factor during the entire host development. This allows to estimate and compare the regulative impact of mortality between different developmental stages, as well as the regulative impact of mortality factors that act on different host stages and their overall impact on host density (ELKINTON et al., 1992).

2 Materials and methods

2.1 Study site

All samples were collected in a mixed oak forest on the “Kalvarienberg” (48°38'20"N, 15°49'40"E) in Eggenburg, at an altitude of approximately 370 m above sea level. Eggenburg is in the northern part of Lower Austria and located in the Pannonian sub-continental climate zone. The climate is warm and arid with an annual precipitation of approximately 500 mm (KILIAN et al., 1994). The predominant tree species is *Quercus petraea*, further tree species are *Quercus cerris*, *Pinus sylvestris*, *Acer platanoides*, *Robinia pseudoacacia*, *Quercus robur*, and *Prunus avium*. It is a sparse high forest with a dense shrub and herb layer, mainly consisting of oaks and grasses. Temperature and light intensity were measured every hour at the study site from April 7th to July 30th, 2020. Daily mean temperature was calculated as the sum of the hourly measurements divided by 24. Further, weather and climate data of the “Zentralanstalt für Meteorologie und Geodynamik” (ZAMG) were used. Particularly data of the ZAMG weather stations Retz (48°46’N, 15°57’E) and Krems (48°25’N, 15°37’E) were considered as they are close to the study site (16 km and 27 km from Eggenburg, respectively), Eggenburg is located in between the two cities, and both are classified into the same forest growth region as Eggenburg (KILIAN et al., 1994). For the precipitation sequence in 2020, data from the station Stift Zwettl (48°37’N, 15°12’E) (46 km distance) were used as no data were available for Retz and Krems.

2.2 Population size of *Lymantria dispar*

The number of gypsy moth egg masses **(Fig. 2)** on trunks and branches up to a height of 4 m on 51 trees was recorded on April 7th, 2020, and the arithmetic mean of egg masses per tree was calculated. For egg masses on higher sections of the trees, discrimination between egg masses deposited in the previous summer (2019) and those deposited in 2018 was difficult, but we attempted to count only intact egg masses as well as possible. Twenty intact egg masses were carefully scraped off the trees into small containers and transferred to the laboratory where the hairs covering the egg masses were removed with paper towel. The number of eggs per egg mass was counted and the proportion of unfertilized eggs was determined optically by stereomicroscopy **(Fig. 3)**. Furthermore, the 20 egg masses were used to examine gypsy moth hatching and egg parasitism.

Fig. 2: Intact brown, spongy egg mass of *L. dispar* on the underside of an oak branch.

2.3 *Lymantria dispar* hatching and egg parasitism

The field-collected egg masses were stored separately in 250 ml plastic boxes at room temperature and examined daily for hatched gypsy moth larvae and emerged egg parasitoids. Hatched individuals were removed daily and the hatching sequence was documented. Egg parasitization rate was calculated for each egg mass as the ratio of the number of parasitoids emerged per number of eggs counted.
Several larvae were reared on artificial diet and used to obtain head capsules from all larval stages. These were later used as a reference for determining the instar of field collected larvae.

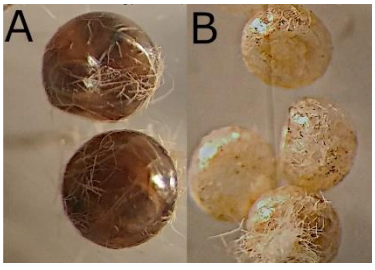

Fig. 3: *L. dispar* eggs in stereomicroscopic view.
A) Dark fertilized eggs.
B) Light and transparent unfertilized eggs.

2.4 Field sampling of *Lymantria dispar* larvae and pupae

Larvae were stage specifically (L1-L6) collected on ten dates between May 14th and July 30th, 2020. A detailed overview is given in **Table 2**. On May 14th, six people were involved in collecting, on all other dates, two people collected for 2-3 hours (except June 23rd with 30 minutes collecting time). Except for instars L2 and L3 – which were the easiest to find – we tried to collect as many individuals as possible on all dates.

To reconstruct the phenology of *L. dispar*, observations on the occurrence of instars at the collection dates were used, supplemented by data from rearing.

To collect L1 and L2 larvae, the branches were hit with a broomstick and the larvae were collected in a beating net **(Fig. 4A)**. Instars L3 and L4 were additionally collected from tree trunks. To collect the final instars (L5 and L6) and pupae, burlap bands were attached to 72 oaks at breast height between June 23rd and July 30th. Burlap bands are used by older larvae as resting places and facilitate collecting in sparse populations **(Fig. 4B-C)**. This technique has already been used in other studies (HOCH et al., 2001; RODEN, 2003).

Fig. 4: Methods for collecting larvae and pupae.
A) Beating net and broomstick.
B) Burlap band trap around the circumference of the trunk at breast height.
C) Larvae resting under burlap band.

Tab. 2: Instar-specific sample sizes and collection dates.

Instar	Date										Total sample size (Σ)
	May		June				July				
	14th	26th	2nd	16th	23rd	30th	7th	16th	21st	30th	
L1	99	-	-	-	-	-	-	-	-	-	99
L2	238	-	-	-	-	-	-	-	-	-	238
L3	-	50	88	11	-	-	-	-	-	-	149
L4	-	-	45	35	4	-	-	-	-	-	84
L5	-	-	-	7	-	26	17	6	8	-	64
L6	-	-	-	-	2	13	12	11	6	2	46
Pupae	-	-	-	-	-	-	2	5	1	4	12
Total (Σ)	337	50	133	53	6	39	31	22	15	6	692
Collection technique	Beating	Beating + Trunk collections		Trunk collections		Burlap bands					

2.5 Laboratory rearing of field-collected *Lymantria dispar* larvae and pupae

Rearing was conducted under semi-field conditions in the insectarium protected from rain and direct sunlight at the Austrian Research Centre for Forests (BFW), in Vienna (48°10'43"N, 16°18'10"E). Walls of the insectarium are permeable to air, so that the temperature roughly corresponded to the field temperature in Vienna.

After collection, the instar of each larva was determined by the width of its head capsule. The occurrence of macrotype tachinid eggs was documented **(Fig. 5)**. The larvae were then transferred to 250 ml plastic boxes **(Fig. 6)**. According to their gregarious lifestyle, L1 and L2 larvae were reared in groups of 10 individuals. From L3 onwards, all larvae were reared individually to prevent the spread of pathogens. Larvae were fed and examined three times a week, and the boxes were cleaned with paper towel soaked in ethanol. The work was carried out with nitrile gloves and tweezers, which were cleaned with ethanol between each larva and box, respectively. Larvae and pupae were reared until death or eclosion of the adult moths. Individuals that successfully developed into adults were classified as "survivors".

Fig. 5: Dead larva, carrying numerous white macrotype tachinid eggs.

Oak leaves (*Quercus* spp.) from the study site were used for feeding. Oak branches were collected at the study site and stored in water, enriched with cut flower additives ("flower food") until the leaves were used. In a few exceptional cases, oak leaves from trees in the Vienna Woods and Norway Maple (*Acer platanoides*) leaves from trees in Vienna (BFW premises) were used, due to their easier availability. *Acer* is listed as a preferred host plant of *L. dispar* (WELLENSTEIN and SCHWENKE, 1978) and the literature shows no effect on mortality from temporary diet changes (STOYENOFF et al., 1994). Larvae in the rearing boxes accepted maple leaves without any problems.

Fig. 6: Plastic rearing boxes with fresh oak leaves as food for larvae.

2.6 Determination of mortality

2.6.1 Mortality by parasitoids

Death by parasitization was assumed if one or more parasitoids emerged from any host stadium. In case of hymenopteran parasitoids, the number of cocoons per host (solitary/gregarious) and the cocoon colour were the first identification features for the genus or species level. Cocoons were stored under semi-field conditions in 2 ml test tubes with an air hole and regularly checked for adult emergence. Adult parasitoids were sexed and identified using the key of SIMONS et al. (1979). The sex ratio of *A. disparis* was determined on a subsample of 100 individuals.

Fig. 7: Marginal bristles on the third abdominal segment of an adult *B. pratensis* tachinid fly.

For the determination of dipteran parasitoids, distinctive traits in the posterior part of the puparium were used, particularly the spiracular plates and their extensions, as described by ZÚBRIK (1998) **(Fig. 8)**. Subsequently, the puparia were stored just like hymenopteran cocoons. In October 2020, all tachinid puparia were dissected and pharate adult specimens of *Blepharipa* sp. inside the puparia were used to distinguish the two

species *B. pratensis* and *B. schineri*, based on the presence (*B. pratensis*) or absence (*B. schineri*) of median marginal bristles on the fused abdominal segment 1+2 and on segment three **(Fig. 7)** (SABROSKY and REARDON, 1976).

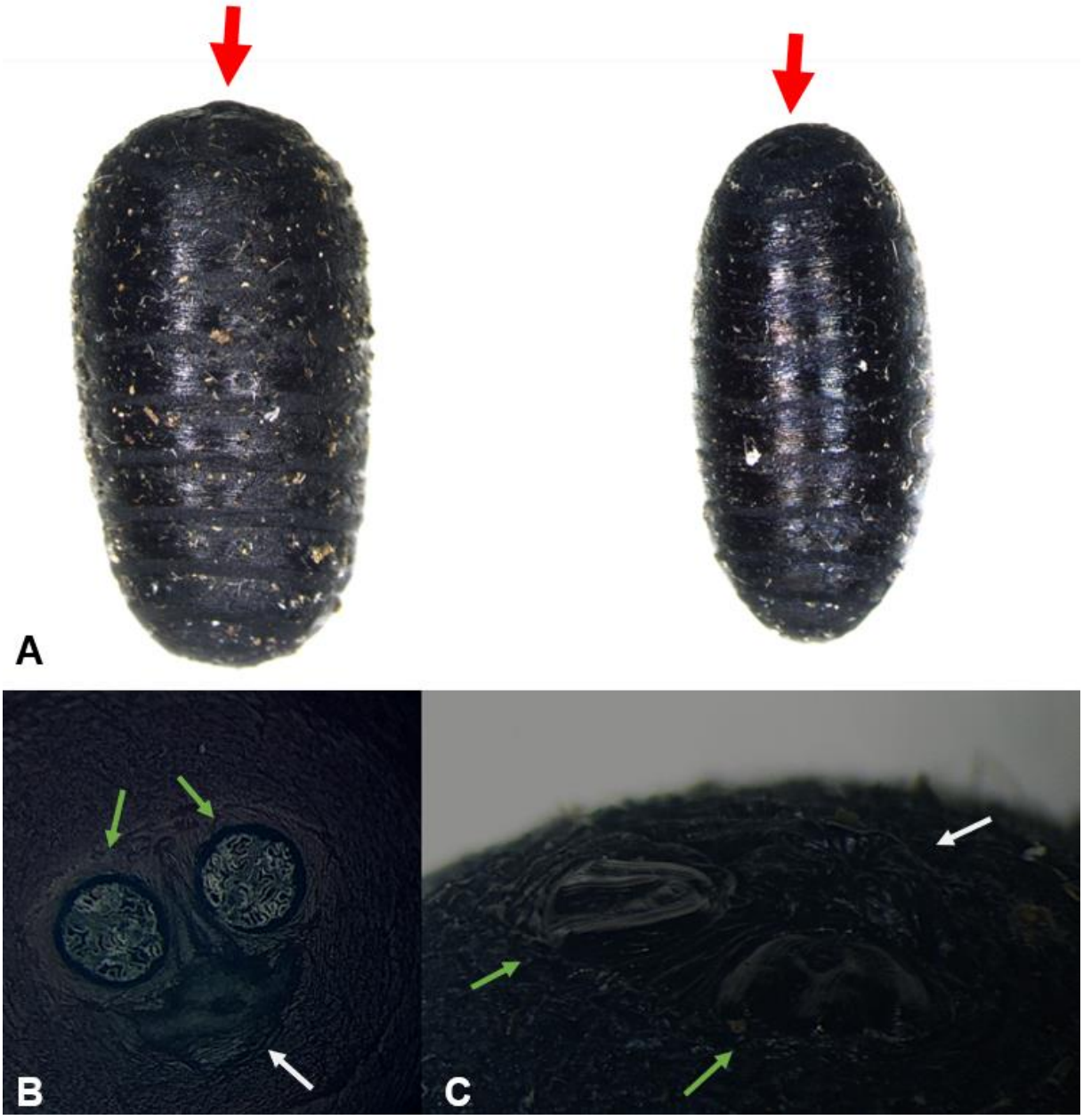

Fig. 8: Distinctive traits of tachinid puparia used for determination.
A) Slightly conical shaped puparium of *Blepharipa* sp. (left) and regularly shaped puparium of *Parasetigena silvestris* (right). The position of the spiracular plates and their extensions is marked by the red arrows.
B) *Blepharipa* sp.: The whole surface of the spiracular plates (green arrows) is covered with irregular meandric lines. The subspiracular appendix (white arrow) is conspicuously raised.
C) *Parasetigena silvestris*: The spiracular plates (green arrows) are covered with three broad and nearly straight furrows. The subspiracular appendix (white arrow) is hardly raised.

2.6.2 Mortality by pathogens

Dead larvae with no apparent signs of parasitism were transferred to 2 ml test tubes. If disintegration had already started, the tubes were immediately frozen to -18 °C. When the cadaver was in better condition, it was stored under semi-field temperatures for 2-3 days, to allow parasitoids to egress the host or pathogens to multiply. According to the protocol of BLACKBURN and HAJEK (2018), a wet piece of paper towel was placed in the tube to promote the formation of *E. maimaiga* conidia and facilitate their subsequent detection. After 2-3 days the larvae were examined again for the emergence of parasitoids; if no parasitoids appeared, they were transferred to -18 °C for storage until the final examination.

After the emergence of adult moths or parasitoids had ended, all dead larvae and pupae with no apparent signs for parasitization were dissected and examined for pathogens using phase contrast microscopy. From big cadavers, a small sample of fat body, mid gut, and silk gland tissue was taken from the anterior third of the body and transferred into a drop of water on a microscope slide. From small larvae, only the head capsule was removed and the remaining parts were transferred to the slide. The sample was quenched with a cover slip in a circular motion to leave a thin, smeared layer and examined with a 200-400x magnification under phase contrast microscopy. Occlusion bodies of NPV and spores of *Entomophaga maimaiga* were identified based on the description by BLACKBURN and HAJEK (2018). Microsporidia were identified by G. HOCH and the identification was confirmed with a bioassay. Samples with a high density of fungal spores other than *E. maimaiga* were classified as killed by unknown fungi. Not all species found in the samples are likely to be entomopathogenic.

If no viral, fungal, or microsporidian structures were found, or the sample contained only a small number of pathogen-like structures, but no clear finding was possible, the individual was classified in the group of unknown mortality. Bacteria were not assumed the primary cause of death due to their rapid development after the death of the host. Therefore, samples with a high bacterial density were classified as unknown mortality when no other pathogen was present.

2.7 Statistical evaluation of *Lymantria dispar* mortality

The mortality rates presented in this study represent the stage-specific apparent mortality of the collected individuals. These rates were calculated as the proportion of individuals killed by a mortality factor out of all individuals collected in a given stage. The significance of the differences in mortality rates between the sample groups was tested using Fisher's exact test.

In addition, a second statistical approach was used to partially separate the influence of simultaneously acting mortality factors and to estimate the generation effect of individual mortality factors. For this purpose, stage-specific marginal infestation rates (m) and k-values (k) were determined, using a method based on ELKINTON et al. (1992) but modified in some points. While marginal infestation rates and k-values are usually only calculated for parasitoids, in the present calculation no differentiation was made between parasitoids, pathogens, and unknown mortality.

2.7.1 Marginal infestation rate

Marginal infestation rates were derived stage-specifically for each mortality factor (*m*) from apparent mortality rates (*d*) and the theoretical value *c*, as shown in **Equation 4**. Calculations were based on the procedure suggested by ELKINTON et al. (1992). In order to avoid double counting, the apparent mortality (*d*) was assumed for these calculations as the proportion of larvae killed in stage n to all larvae collected in stage n **(Eqs. 1 and 2)**.

Eq. 1:
$$d_{A\text{-}n} = \frac{\text{individuals collected and killed in stage n by factor A}}{\text{individuals collected in stage n}}$$

Eq. 2:
$$d_{B\text{-}n} = \frac{\text{individuals collected and killed in stage n by all other factors}}{\text{individuals collected in stage n}}$$

An exception was made for the larval-pupal parasitoid *B. pratensis*, as the small sample size of field-collected pupae would not have allowed a reliable evaluation. In this case, pupae of all larvae collected in their final instar and inidviduals collected as pupae in the field were added to form the denominator. Those among them, which were killed as pupae, formed the numerator in **Eqs. 1 and 2**.

Equation 3 is an intermediate step to simplify the expression of **Eq. 4**.

Eq. 3:
$$b = c * (d_{A\text{-}n} + d_{B\text{-}n}) + 1 - d_{B\text{-}n}$$

Equation 4 shows the calculation of the marginal infestation rate of factor A in host stage n.

Eq. 4:
$$m_{A\text{-}n} = \frac{b - \sqrt{(b^2 - 4 * c * d_{A\text{-}n})}}{2 * c}$$

c is a measure for the competitiveness of a mortality factor and describes the probability that factor A will be scored as apparent mortality agent of a host that has been co-infested with another mortality agent. Ideally, *c* should be determined experimentally for every possible combination of mortality factors but this is hardly possible in field experiments examining multiple factors (ELKINTON et al., 1992).

GOULD (1990) tested every single factor (A) against all other combined factors (B), assumed $c = 0.5$ for all interactions, and observed only one small error caused by this estimate. $c = 0.5$ means that if a host individual is infested by factor A and another factor, the probability that factor A is rated as the apparently decisive mortality factor is 50 %. Accordingly, with $c = 0.1$, factor A would appear to be decisive only in 10 % of cases.
I adopted the approach of GOULD (1990) to test each factor (A) against all the others together (B); but instead of assuming $c = 0.5$ for all interactions, I adapted *c* for each individual interaction with a simple indicator.

The time from host infestation to host death and the sequence of infestations of already infested hosts were assumed to be the main determinants of competitive advantages (see

Tab. 3: Adjustment of the calculation factor *c*, depending on the average time from host collection to host death caused by factor A (t_A) or other mortality factors (t_B).

proportion t_A:t_B				*c*
t_A	≥	5 x	t_B	**0.1**
t_A	≥	3 x	t_B	**0.2**
t_A	≥	2 x	t_B	**0.33**
t_A	≥	1.5 x	t_B	**0.4**
t_A		≈	t_B	**0.5**
t_A	≤	0.67 x	t_B	**0.6**
t_A	≤	0.50 x	t_B	**0.67**
t_A	≤	0.33 x	t_B	**0.8**
t_A	≤	0.20 x	t_B	**0.9**

Chapter 1.7). Unfortunately, the sequence of infestation in field-collected larvae is never known. Considering this, I assumed that mortality factors which killed their host fast were competitive superior to mortality factors which killed the host slower. The average time between host collection and host death was compared between the factor in question (t_A) and all other mortality factors (t_B) in the same stage and this was used as an indicator to adjust *c* for each interaction, as described in **Table 3**. The assumption was that the longer it takes a mortality factor A to kill its host, the higher the probability that the host will be killed by a competitive factor with a shorter developmental time before factor A has completed its development. For egg mortality, $c = 0.5$ was assumed.

2.7.2 Killing power

$s_{A\text{-}n}$ describes the probability for a host individual to survive the influence of factor A in stage n, without all other mortality factors. $s_{A\text{-}n}$ is derived from marginal infestation rates (**Eq. 5**) and was further used to calculate the killing value (*k*), as shown in **Eq. 6**.

Eq. 5: $$s_{A\text{-}n} = 1 - m_{A\text{-}n}$$

Eq. 6: $$k_{A\text{-}n} = -\log_{10}(s_{A\text{-}n})$$

Stage specific values of *s* were used to calculate the probability of a host individual to survive the influence of factor A during its total development in the absence of other mortality factors (**Eq. 7**), and the probability of an individual to survive the influence of all mortality factors combined during its development from the egg stage to the adult moth (**Eq. 8**).

Eq. 7: $$S_A = s_{A\text{-egg}} * s_{A\text{-L1}} * s_{A\text{-L2}} * s_{A\text{-L3}} * s_{A\text{-L4}} * s_{A\text{-L5}} * s_{A\text{-L6}} * s_{A\text{-pupae}}$$

Eq. 8: $$S_{A*B} = s_{A*B\text{-egg}} * s_{A*B\text{-L1}} * s_{A*B\text{-L2}} * s_{A*B\text{-L3}} * s_{A*B\text{-L4}} * s_{A*B\text{-L5}} * s_{A*B\text{-L6}} * s_{A*B\text{-pupae}}$$

Similarly, killing powers were subsumed factor-specifically (**Eq. 9**).

Eq. 9: $$K_A = k_{A\text{-egg}} + k_{A\text{-L1}} + k_{A\text{-L2}} + k_{A\text{-L3}} + k_{A\text{-L4}} + k_{A\text{-L5}} + k_{A\text{-L6}} + k_{A\text{-pupae}}$$

2.8 Evaluation of hyperparasitism

Several primary parasitoids of *Lymantria dispar* are strongly affected by hyperparasitoids, which are parasitoids that attack other parasitoids (MUESEBECK and DOHANIAN, 1927; EICHHORN, 1996). In this study, hyperparasitism was examined for the most common primary parasitoids *Glyptapanteles porthetriae*, *Parasetigena silvestris,* and *Blepharipa pratensis*. The presence of hyperparasitoids in the puparia of *P. silvestris* and *B. pratensis* was checked by dissections in October 2020. Identification was done with the key of OEHLKE, 1969.

In the case of *G. porthetriae*, 45 cocoons were collected at the study site on May 28th (eight cocoons) and June 2nd (37 cocoons). The cocoons were kept in a 250 ml plastic box for each collection date and emerging wasps were counted and removed three times a week. Hyperparasitoids were determined by the author with different keys at family (OEHLKE, 1969) and subfamily (SIMONS et al., 1979; BROAD, 2011) level.

3 Results

3.1 Population density, phenology of *L. dispar*, and weather conditions

3.1.1 Population density in spring 2020 and phenology of *L. dispar*

On April 7th, 2020, an average of 13 intact egg masses per tree were counted, with values ranging from 0 to 58. Each egg mass contained 222 (58-640) eggs **(Fig. 9)**. Hatching began on April 7th and L1 larvae were observed until the end of May, seven weeks after the first larvae hatched. L2 larvae were observed until early June, approximately two months after the start of the egg hatching. Larval development accelerated in June with L3 instars **(Fig. 10)**. Of the larvae reared under semi-field conditions, pupae were observed from the end of June until the end of August, 89 % of the larvae pupated in July. Moth eclosion started in the mid of July and roughly the same proportion of adults emerged in July and August. Male moth eclosion peaked on July 27th; the peak of female eclosion was on August 3rd.

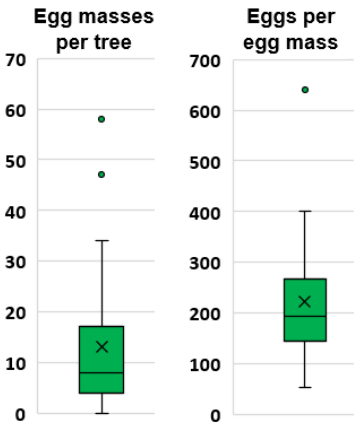

Fig. 9: Egg mass counts of 51 trees and egg counts of 20 egg masses.

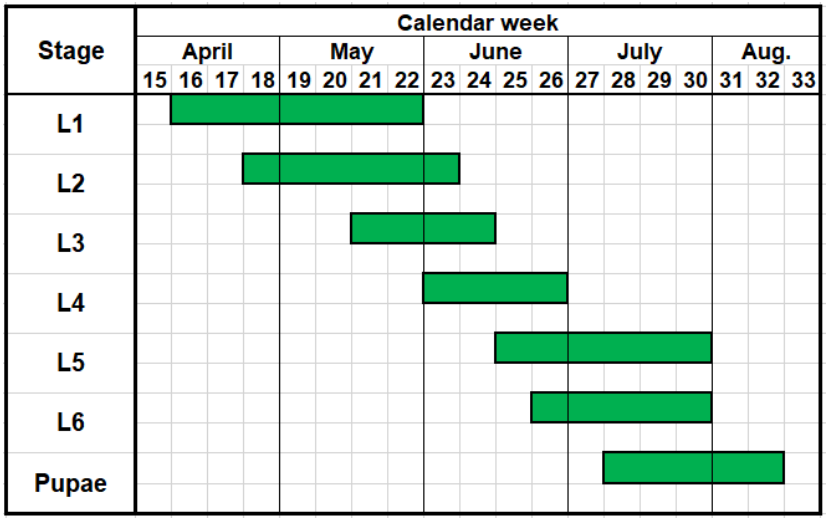

Fig. 10: Phenological sequence of *L. dispar* at the study site Eggenburg in 2020.

3.1.2 Weather conditions

Temperature

The end of march and the first week of April 2020 were characterized by a cold spell in eastern Austria. On April 2nd, the ZAMG station Krems measured a new record low for April of -5.0 °C (ZAMG, 2020c). The temperature measurements on the study site started on April 7th. On April 15th, frost temperatures with a minimum of -0.8 °C were measured for five hours. Apart of that, the last three weeks of April were unusually warm with an average daily mean temperature of 12.5 °C. In May (12.5 °C), the average daily temperature did not exceed the value in April and the average daily maximum in May was lower than in the last three weeks of April. From June, temperature generally increased continuously, until mid-July was followed by a cool period with daily mean temperatures of around 15 °C over eight days **(Fig. 11)**. The measured daily mean temperatures were 16.8 °C in June and 18.5 °C in July.

The monthly mean temperature in Lower Austria in April was about 1.5 °C above the long-term mean (ZAMG, 2020c), in May 1.2 °C below the average (ZAMG, 2020d), in June +0.7 °C (ZAMG, 2020e), and in July +0.5 °C (ZAMG, 2020f).

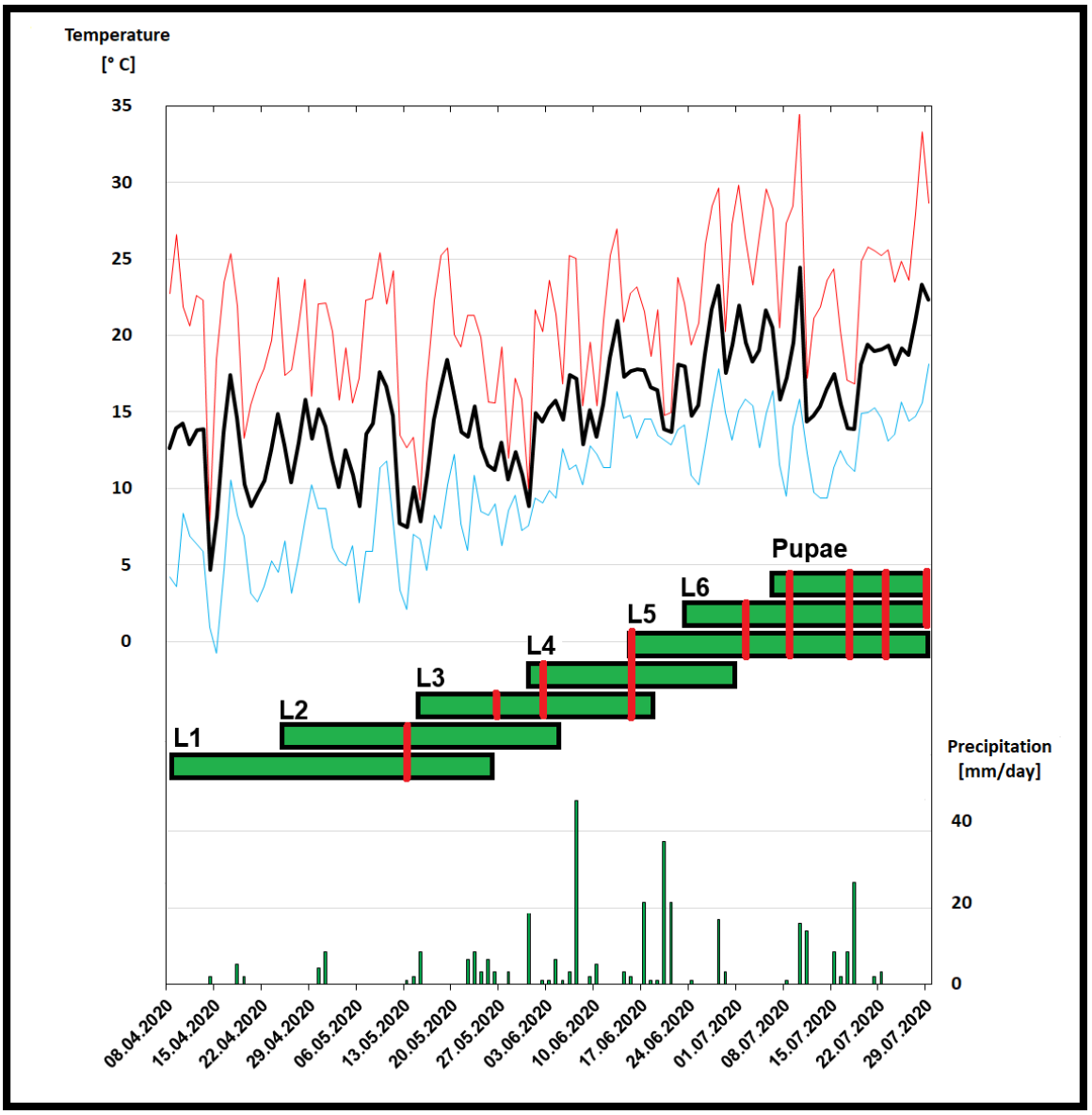

Fig. 11: Measured temperature at the study site, precipitation data from the weather station "Stift Zwettl" (ZAMG, 2020a), and phenology of *L. dispar*.
Red markings within the phenology bars represent collection dates with the corresponding stages collected on each date.

Precipitation

Precipitation data were not measured at the study site; however, some clear trends can be deduced from publicly available data. Spring 2020 was extraordinarily dry in Lower Austria with precipitation deficits of -40 % in March (ZAMG, 2020b) and -66 % in April (ZAMG, 2020c). In Retz, 42 mm of precipitation was measured within these two months, in Krems only 21 mm (ZAMG, 2020a). The dry period ended in the second half of May **(Fig. 11)**, resulting in a precipitation amount close to the long-term average in this month (ZAMG, 2020d). June was relatively humid with 61 % higher rainfall in Lower Austria (ZAMG, 2020e) and the heaviest rainfall event during the period of larval development was reported on June 7th **(Fig. 11)**. July again corresponded to the long-term average (ZAMG, 2020f).

3.2 Larval hatching and egg mortality

3.2.1 Larval hatching

Hatching of the larvae was observed on 20 egg masses, collected on April 7th, 2020 and stored at room temperature. The first larvae began to hatch on the evening of the collection day. The peak of hatching was observed on April 9th, more than 90 % of the larvae hatched within one week after sampling, and the last larva hatched on April 22nd. Although only 1.0 % of the eggs were visually identified as unfertilized, only 2,356 larvae hatched from 4,433 eggs, which corresponds to 53.2 % hatching rate. The hatching rates for individual egg masses were between 0 % and 85.8 % **(Fig. 12)**. No correlation was found between the number of eggs and the hatching rate of the individual egg masses (Pearson coefficient: r = -0.07).

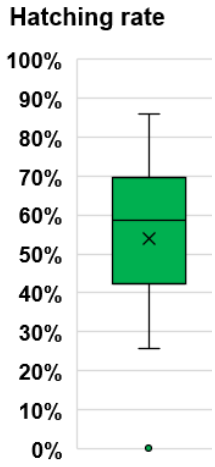

Fig. 12: Larval hatching rate for 20 *L. dispar* egg masses.

3.2.2 Egg parasitization by *Anastatus disparis*

On April 29th, the emergence of eight parasitic wasps was observed from a gypsy moth egg mass. On the following 19 days, a few more individuals were observed and a total of 46 individuals emerged by May 17th. From May 18th, the emergence rate of parasitoids increased dramatically, peaking on May 23rd with 97 individuals emerging within 24 hours. At the beginning of June, the numbers decreased quickly and on June 8th the last individuals emerged.

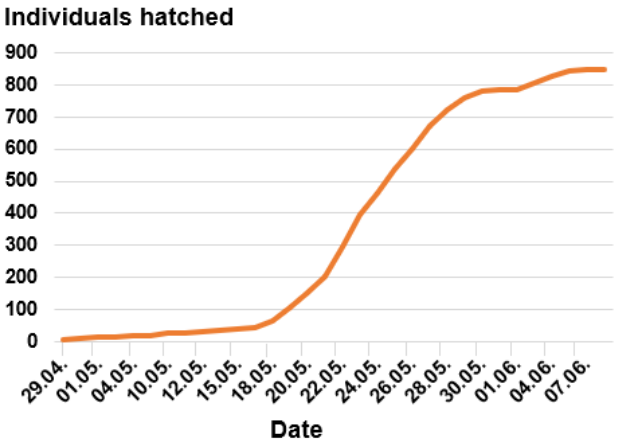

Fig. 13: Cumulative emergence of *A. disparis* wasps from 20 *L. dispar* egg masses (4,433 eggs), stored at room temperature.

The emergence of the adult wasps extended over a period of 41 days **(Fig. 13)** and showed a unimodal distribution for the entire sample. The emergence time was significantly longer than that of *L. dispar* larvae, but hatching data cannot be related to the field, as the egg masses were stored at room temperature. At the level of the individual egg masses, emergence extended over 10 to 39 days and in many cases showed irregular and multimodal distributions. The number of wasp individuals that emerged within 24 hours ranged from one (or in many cases zero) to 19. The sex ratio of the wasps was 65:35 (females:males).

A total of 849 specimens of *Anastatus disparis* **(Fig. 16)** were obtained from 4,433 *L. dispar* eggs, which corresponds to an egg parasitization rate of 19.2 %. With the exception of two egg masses, from which neither *L. dispar* nor *A. disparis* emerged, all the other 18 egg masses were parasitized by 2.8 % to 46.0 % **(Fig. 14)**. As with larvae hatching, no linear relationship was found between the number of eggs and the parasitization rate of the individual egg masses (Pearson coefficient: r = 0.05).

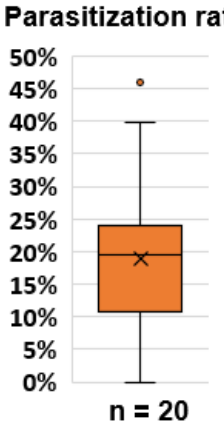

Fig. 14: Parasitization rates from *A. disparis* for 20 *L. dispar* egg masses.

After the emergence of *A. disparis* 27.7 % eggs remained, from which neither *L. dispar* nor *A. disparis* emerged. The proportion of these eggs ranged between zero and 100 % for individual egg masses **(Fig. 15)**.

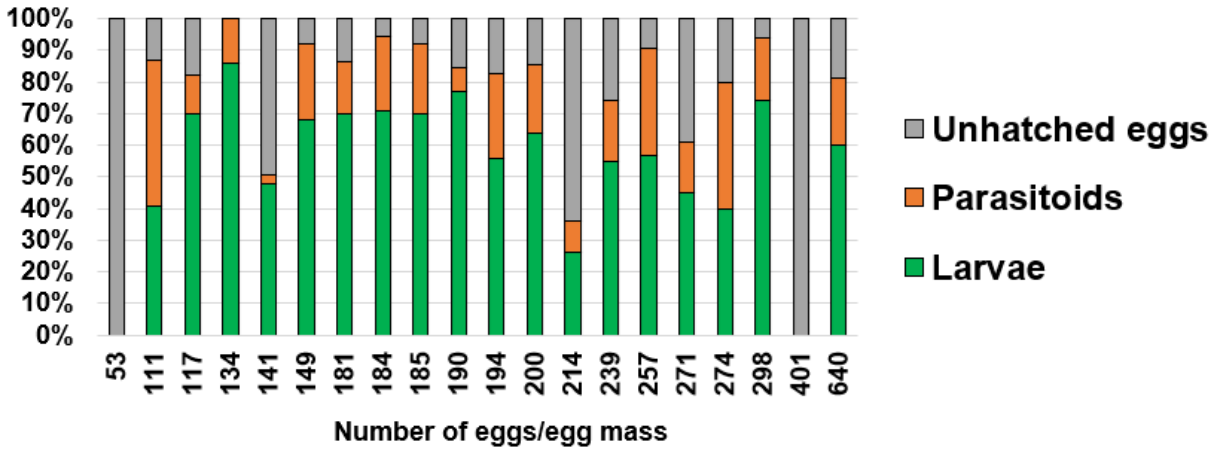

Fig. 15: Cumulative hatching rates of *L. dispar* larvae and emergence of *A. disparis* wasps from 20 *L. dispar* egg masses.

Fig. 16: Adult wasps of *Anastatus disparis*.
A) Female.
B) Male.
C) Wasp emerging from an egg of *L. dispar*.

3.3 Mortality of young larvae (L1 and L2 instars)

Total mortality rates were very similar for larvae collected in the first and second instar, but the main causes of death were different. While L1 larvae suffered high pathogen mortality and parasitoids were of minor importance, the roles of both groups of natural enemies changed in the opposite in larvae collected as second instars. The unknown mortality reached similarly high values in both groups **(Fig. 17)**.

The braconid wasp *G. porthetriae* was the dominant parasitoid species in both sample groups and responsible for 81 % of the apparent parasitism of young larvae (L1 and L2), while the ichneumonid wasp *Hyposoter tricoloripes* and the tachinid fly *Blepharipa pratensis* were observed at low prevalence. Among pathogens, the nuclear polyhedrosis virus (NPV) dominated in both sample groups, although the dominance was much stronger in the first instar **(Fig. 17)**.

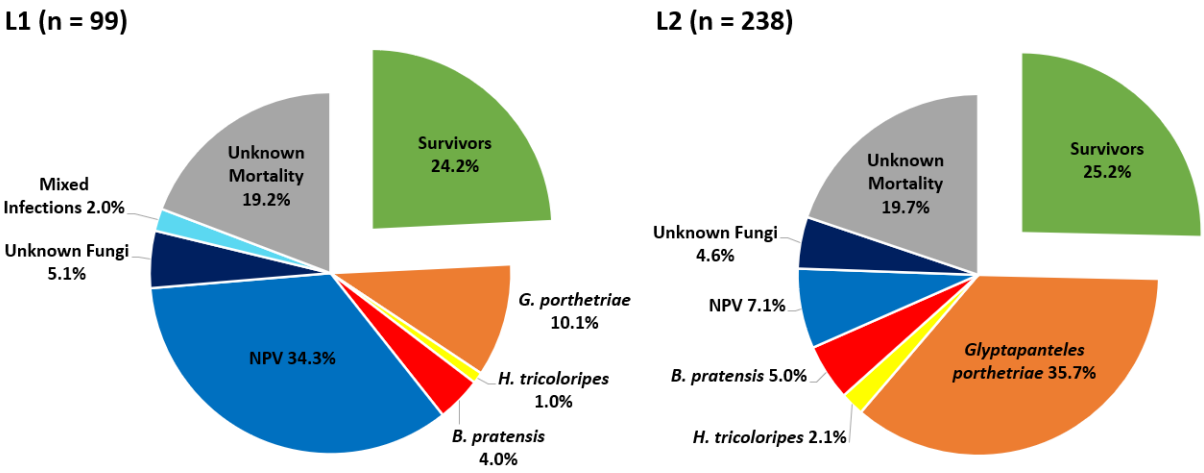

Fig. 17: Apparent mortality rates and mortality causes for *L. dispar* larvae collected as first and second instars.

3.3.1 Mortality of L1 larvae

NPV caused nearly half of the total mortality observed in larvae collected as first instars. Cadavers of five larvae contained considerable amounts of spores of unidentified fungal species and in two cases NPV occlusion bodies and unidentified fungal spores were found within one cadaver. Of the L1-collected larvae killed by pathogens, 51 % died in their first instar, another 39 % died in the second instar. Pathogen-induced mortality occurred on average on day 16 post collection. Death from unknown causes occurred predominantly within the first two instars and took 16 days on average. Two larvae collected in the first instar died as pupae more than 90 days after collection.

No parasitoids emerged from first instar hosts. For *G. porthetriae*, death occurred in the second (70 %) or third instar (30 %), on average 18 days after collection. One individual was killed by *H. tricoloripes* as L3 larva, 34 days post collection. Four larvae collected as L1 were killed by *B. pratensis* in the pupal stage, in these cases 74-90 days passed from collection to death.

3.3.2 Mortality of L2 larvae

Pathogen mortality was significantly lower in larvae collected in the second instar compared to L1-collections ($p < 0.001$). Pathogens accounted for 16 % of the total mortality in this group. NPV caused 61 % of the total pathogen mortality, 39 % was caused by unidentified fungi. With one exception, larvae killed by fungi died in the second instar, on average 17 days after collection. The majority of the larvae killed by NPV died in the third instar, on average 29 days after collection. Death from unknown causes occurred almost exclusively in the second or third instar, on average 17 days after collection.

Parasitoids were the most common cause of death of larvae collected as second instars. Among the parasitoids, *G. porthetriae* dominated strongly and was responsible for more than 80 % of the apparent parasitoid mortality in this sample group. Both total parasitization rate and parasitization rate by *G. porthetriae* exceeded the values for L1-collected larvae significantly ($p < 0.001$). In the case of *G. porthetriae,* host death occurred predominantly in the second instar, on average nine days after collection. Death from *H. tricoloripes* occurred in the third instar after 20-32 days. Hosts parasitized by *B. pratensis* always died as pupae, on average 80 days after collection.

3.4 Mortality of middle-aged larvae (L3 and L4 instars)

Larvae collected as instars L3 and L4 showed the lowest overall larval mortality rate; particularly pathogens and unknown causes of death were observed with low prevalence in this group. Considering these mortality agents, the occurrence of *E. maimaiga* in L4 larvae was the most striking difference between the two instars.

G. porthetriae was again the most important mortality factor, but less dominant than in young larvae. An increased diversity of parasitoid species resulted in a higher total parasitization rate for middle-aged larvae than in the first two instars **(Fig. 18)**.

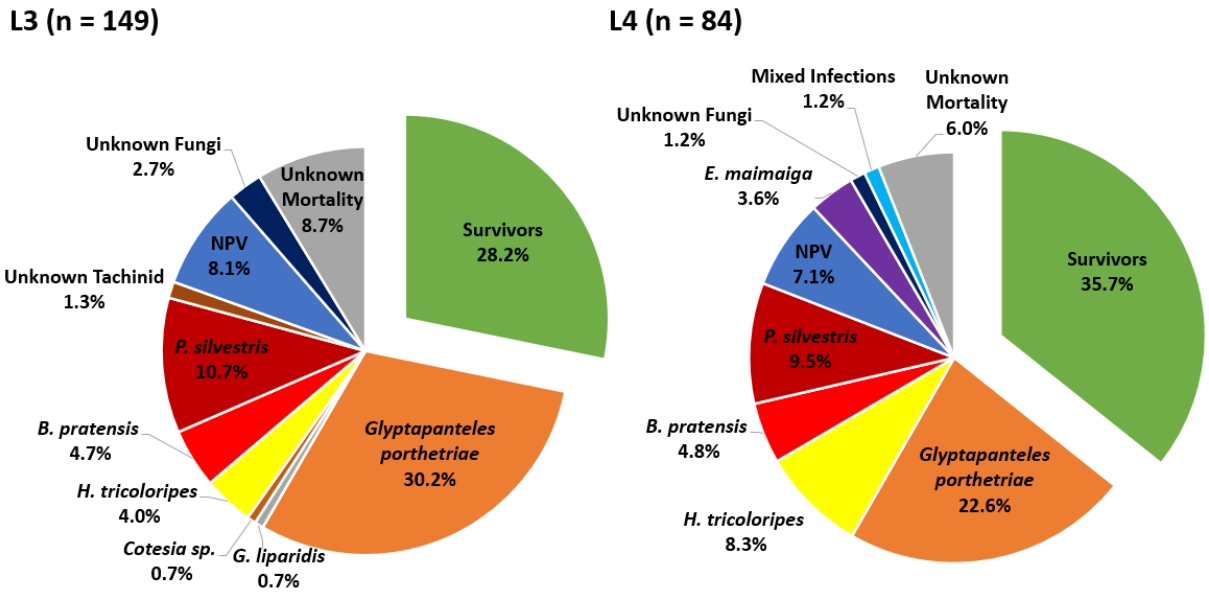

Fig. 18: Apparent mortality rates and mortality causes for *L. dispar* larvae collected as third and fourth instars.

3.4.1 Mortality of L3 larvae

Pathogen mortality was lowest in larvae collected in the third instar. NPV was responsible for 75 % of the pathogen mortality, killing hosts in the L3 or L4 stage, on average 34 days after collection. Unknown causes of mortality became manifest more quickly, on average after 20 days and occurred predominantly in the stage of collection.

All six larval parasitoid species found in this study were represented in larvae collected in the third instar. Parasitism accounted for 73 % of the mortality within this group. Parasitization rates increased considerably with later collection dates. L3 larvae collected on May 26th showed a parasitization rate of 34 %, while 58 % of L3 larvae collected on June 2nd were apparently parasitized. Of the L3 larvae collected on June 16th, ten of eleven larvae (91 %) were killed by parasitoids. This was mainly driven by a significant (χ^2-test: $p < 0.001$) increase in the prevalence of *G. porthetriae* with parasitization rates of 18 %, 31 %, and finally 82 %. Correspondingly, the overall mortality also varied greatly depending on the collection date and ranged from 56 % (May 26th) to 91 % (June 16th).

Sixty percent of the parasitoid mortality in this group was caused by braconids, 32 % by tachinids. Among braconids, *G. porthetriae* dominated strongly. *Glyptapanteles liparidis* and *Cotesia* sp. were each obtained from only one host individual. Regarding tachinids, *B.*

pratensis showed no higher parasitization rates than in L1 and L2 larvae. A different pattern was observed for *P. silvestris*. While macrotype eggs of *P. silvestris* were hardly observed in young larvae, more than a third of the L3 larvae were carrying tachinid eggs at the time of collection **(Fig. 27)**. The apparent mortality from *P. silvestris* reached 10.7 %. A further 1.3 % of the L3-collected larvae were killed by tachinid maggots, which egressed the host larvae but did not pupate successfully and therefore could not be determined (shown as "unknown Tachinid" in **Fig. 19** and **Tab. 4**). Since they killed the host in the larval stage, it is likely they were also specimens of *P. silvestris*.

3.4.2 Mortality of L4 larvae

In addition to the low prevalence of NPV and unidentified fungi, also *E. maimaiga* caused pathogen mortality in larvae collected in the fourth instar. In three larvae, the fungus was assumed to be the sole cause of death, one host larva showed a mixed infection by NPV and *E. maimaiga*. While death from NPV took on average 26 days after collection, larvae killed by *E. maimaiga* died within three to eight days.

Compared to L3 larvae, the relative importance of braconids decreased to 50 % of the parasitoids. However, this was exactly compensated by ichneumonids, their relative importance increased to 18 %. Tachinids accounted for 32 % of parasitoid mortality, and the apparent mortality rates of *P. silvestris* and *B. pratensis* were very similar to those of L3 larvae.

With 64.3 %, larvae collected in the fourth instar showed the lowest overall larval mortality rate. The mortality rate was particularly low in the L4 larvae collected on June 2nd (51 %) and increased significantly ($p = 0.006$) to 80 % in larvae collected in mid and late June. This was mainly driven by increasing mortality from the hymenopteran parasitoids *G. porthetriae* and *H. tricoloripes*. *Glyptapanteles porthetriae* caused 9 % apparent mortality in the larvae collected on June 2nd and 38 % in the larvae collected on June 16th and 23rd. An opposite pattern was observed for tachinids, which caused 23 % of the apparent mortality in the sample from June 2nd, compared to 6 % at the later collection dates in June. Thus, tachinids accounted for 64 % of parasitoid mortality in L4 larvae collected in early June and 11 % in larvae collected in mid and late June.

3.5 Mortality of old larvae (L5 and L6 instars)

Old larvae suffered a very high mortality from parasitoids, which was almost exclusively caused by the two tachinid species *P. silvestris* and *B. pratensis*. Compared to middle-aged larvae, the pathogen mortality in old larvae increased again. The species diversity among pathogens was highest in the old larvae. *Entomophaga maimaiga* peaked in prevalence in this group **(Fig. 19)**.

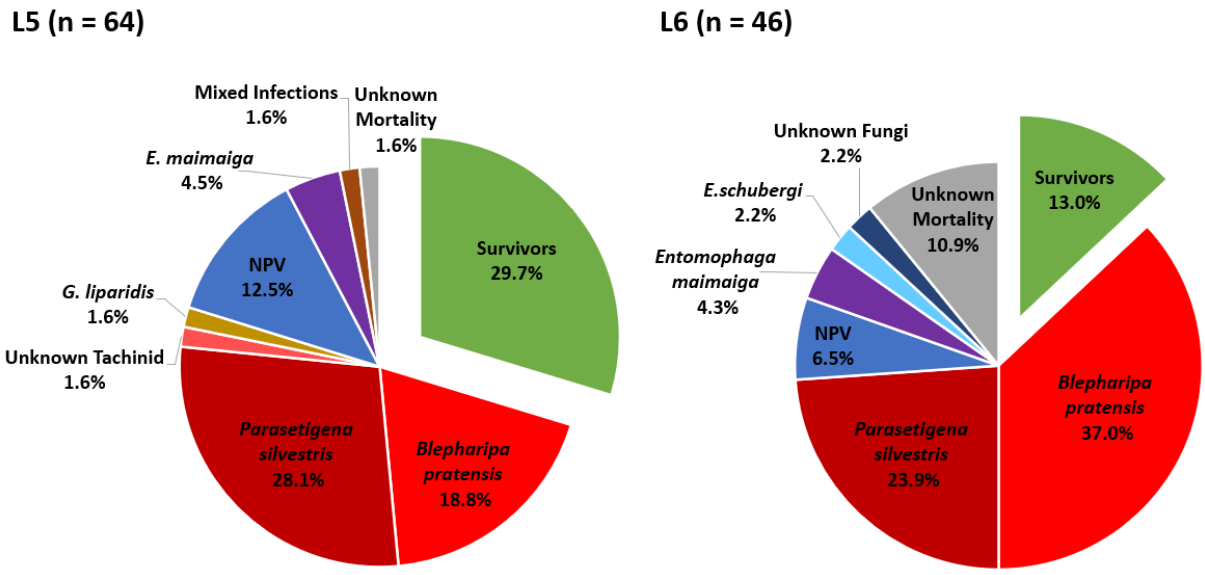

Fig. 19: Apparent mortality rates and mortality causes for *L. dispar* larvae collected as fifth and sixth instars.

3.5.1 Mortality of L5 larvae

Except for L1 larvae, the highest level of apparent pathogen mortality (18.8 %) was observed in larvae collected in the fifth instar. NPV clearly dominated the pathogens and was involved in 76 % of the pathogen mortality. *E. maimaiga* was most frequently observed within this group. One larva showed a mixed infection by NPV and *E. maimaiga*. All L5 larvae killed by pathogens died in the stage of collection. Death occurred quickly after the collection, on average six days passed until death.

Parasitoid mortality was caused almost exclusively by tachinids. One host individual was killed by the braconid *G. liparidis*. Among the tachinids, *P. silvestris* dominated moderately and reached the highest mortality rate in this group with roughly 30 % apparent mortality. One tachinid maggot killed the host but did not pupate successfully (shown as “unknown Tachinid” in **Fig. 19** and **Tab. 4**). Again, it is assumed that it was a specimen of *P. silvestris*.

There were no significant differences in overall mortality, total parasitization rate or pathogen mortality in the L5 larvae collected in June and July, respectively. *Parasetigena silvestris* was found more frequently in larvae collected in June with a rate of 36.4 % of apparent mortality, compared to 19.4 % in the larvae collected in July. However, the difference was not significant ($p = 0.108$). *B. pratensis* was slightly more frequent in the larvae collected in July, but again the difference was not significant ($p = 0.329$).

3.5.2 Mortality of L6 larvae

The highest overall mortality (87 %) was observed in L6 larvae and the overall mortality was significantly higher than that of the larvae collected in the fifth instar ($p = 0.032$). While parasitoid and pathogen mortality did not differ between L5 and L6 larvae, the unknown mortality was significantly higher in larvae collected in the sixth instar ($p = 0.045$).
Four out of five larvae killed by unknown factors died the day after collection. One individual died as pupa.

The NPV mortality decreased compared to L5 larvae, but not significantly (p = 0.174). *Entomophaga maimaiga* occurred in a similar prevalence (4.5 %). One individual was apparently killed by the microsporidium *Endoreticulatus schubergi*.

With 60.9 %, total parasitism was the highest of all larval stages. *Parasetigena silvestris* and *B. pratensis* accounted for 70 % of the total mortality in this group. Apparent mortality by *P. silvestris* decreased slightly compared to L5 larvae, while the parasitization rate of *B. pratensis* increased significantly (p = 0.028) from 18.8 % (L5) to 37.0 % (L6). While *B. pratensis* accounted for 38 % of the parasitoid mortality in L5-collected larvae, this value increased to 61 % in larvae collected in the sixth instar.
No significant differences in terms of the collection date were observed for any mortality factor in this group.

3.6 Pupal mortality

Despite an intensive search for pupae, only twelve gypsy moth pupae were found in the field. Due to this small sample size, statements to pupal mortality in the field are of little significance. Total mortality of the field-collected pupae was 41.7 %. Two individuals (16.7 %) were killed by *B. pratensis*, three individuals (25.0 %) died for unknown reasons.
No apparent damage to the pupae by predators was observed in the field.

Tab. 4: Apparent mortality rates and mortality causes of larvae and pupae based on the collection stage.

Cause of mortality	L1 n = 99		L2 n = 238		L3 n = 149		L4 n = 84		L5 n = 64		L6 n = 46		Pupae n = 12			L1+L2 n = 337		L3+L4 n = 233		L5+L6 n = 110		Total n = 692	
	%	n	%	n	%	n	%	n	%	n	%	n	%	n		%	n	%	n	%	n	%	n
Parasitism	15.2%	15	43.3%	103	52.3%	78	45.2%	38	50.0%	32	60.9%	28	16.7%	2		35.0%	118	49.8%	116	54.5%	60	42.8%	296
Braconidae	10.1%	10	35.7%	85	31.5%	47	22.6%	19	1.6%	1	--	0	--	0		28.2%	95	28.3%	66	0.9%	1	23.4%	162
Glyptapanteles porthetriae	10.1%	10	35.7%	85	30.2%	45	22.6%	19	--	0	--	0	--	0		28.2%	95	27.5%	64	--	0	23.0%	159
Glyptapanteles liparidis	--	0	--	0	0.7%	1	--	0	1.6%	1	--	0	--	0		--	0	0.4%	1	0.9%	1	0.3%	2
***Cotesia* sp.**	--	0	--	0	0.7%	1	--	0	--	0	--	0	--	0		--	0	0.4%	1	--	0	0.1%	1
Ichneumonidae																							
Hyposoter tricoloripes	1.0%	1	2.1%	5	4.0%	6	8.3%	7	--	0	--	0	--	0		1.8%	6	5.6%	13	--	0	2.7%	19
Tachinidae	4.0%	4	5.0%	12	16.8%	25	14.3%	12	48.4%	31	60.9%	28	16.7%	2		4.7%	16	15.9%	37	53.6%	59	16.5%	114
Parasetigena silvestris	--	0	--	0	10.7%	16	9.5%	8	28.1%	18	23.9%	11	--	0		--	0	10.3%	24	26.4%	29	7.7%	53
Blepharipa pratensis	4.0%	4	5.0%	12	4.7%	7	4.8%	4	18.8%	12	37.0%	17	16.7%	2		4.7%	16	4.7%	11	26.4%	29	8.4%	58
Unknown tachinids	--	0	--	0	1.3%	2	--	0	1.6%	1	--	0	--	0		--	0	0.9%	2	0.9%	1	0.4%	3
Pathogens	41.4%	41	11.8%	28	10.7%	16	13.1%	11	18.8%	12	15.2%	7	--	0		20.5%	69	11.6%	27	17.3%	19	16.6%	115
NPV	34.3%	34	7.1%	17	8.1%	12	7.1%	6	12.5%	8	6.5%	3	--	0		15.1%	51	7.7%	18	10.0%	11	11.6%	80
Entomophaga maimaiga	--	0	--	0	--	0	3.6%	3	4.7%	3	4.3%	2	--	0		--	0	1.3%	3	4.5%	5	1.2%	8
Endoreticulatus schubergi	--	0	--	0	--	0	--	0	--	0	2.2%	1	--	0		--	0	--	0	0.9%	1	0.1%	1
Unknown fungi	5.1%	5	4.6%	11	2.7%	4	1.2%	1	--	0	2.2%	1	--	0		4.7%	16	2.1%	5	0.9%	1	3.2%	22
Mixed infections	2.0%	2	--	0	--	0	1.2%	1	1.6%	1	--	0	--	0		0.6%	2	0.4%	1	0.9%	1	0.6%	4
Unknown Mortality	19.2%	19	19.7%	47	8.7%	13	6.0%	5	1.6%	1	10.9%	5	25.0%	3		19.6%	66	7.7%	18	5.5%	6	13.4%	93
Total mortality	75.8%	75	74.8%	178	71.8%	107	64.3%	54	70.3%	45	87.0%	40	41.7%	5		75.1%	253	69.1%	161	77.3%	85	72.8%	504
Survivors	24.2%	24	25.2%	60	28.2%	42	35.7%	30	29.7%	19	13.0%	6	58.3%	7		24.9%	84	30.9%	72	22.7%	25	27.2%	188

3.7 Larval and larval-pupal parasitoids

Parasitoids were the most frequent cause of mortality and were responsible for 59 % of the total mortality in all larvae and pupae collected in the field. In dependence on the instar, parasitization rates ranged from 15.2 % (L1) to 60.9 % (L6) **(Tab. 4)**.

3.7.1 Braconidae

<u>*Glyptapanteles porthetriae*</u>

Glyptapanteles porthetriae **(Fig. 21)** was the dominant mortality factor of larvae collected in the second, third, and fourth instar. Percent apparent parasitism peaked in larvae collected in the second instar (35.7 %) **(Fig. 20)**. In the first four instars of *L. dispar*, *G. porthetriae* accounted for 50 % (L4) to 83 % (L2) of the parasitoid mortality and 20 % (L1) to 48 % (L2) of the total mortality. The parasitization rate was significantly lower in larvae collected in the first instar than in the larvae collected in the second to fourth instars. Furthermore, a significant difference was found between L2 and L4 larvae ($p = 0.018$), but not between L2 and L3 ($p = 0.157$) and L3 and L4 ($p = 0.137$). However, if differences are taken in account for certain collection dates, the parasitization rates in larvae collected in the third instar were significantly higher than in larvae collected in the fourth instar on June 2nd ($p = 0.003$) and June 16th ($p = 0.018$).

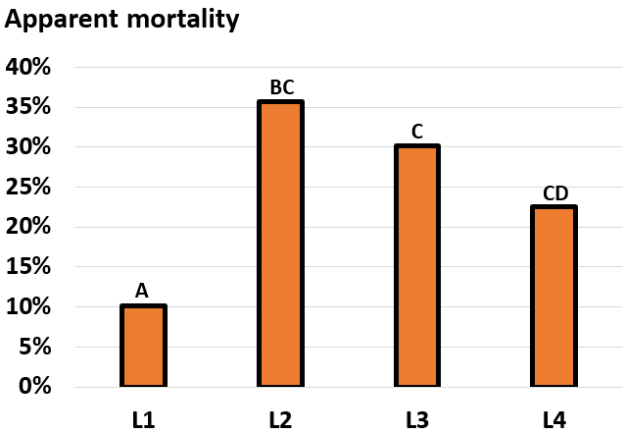

Fig. 20: Apparent mortality caused by *G. porthetriae* based on *L. dispar* collection stages.
Letters above the columns represent statistical significances (Fisher's exact test, $p < 0.05$).

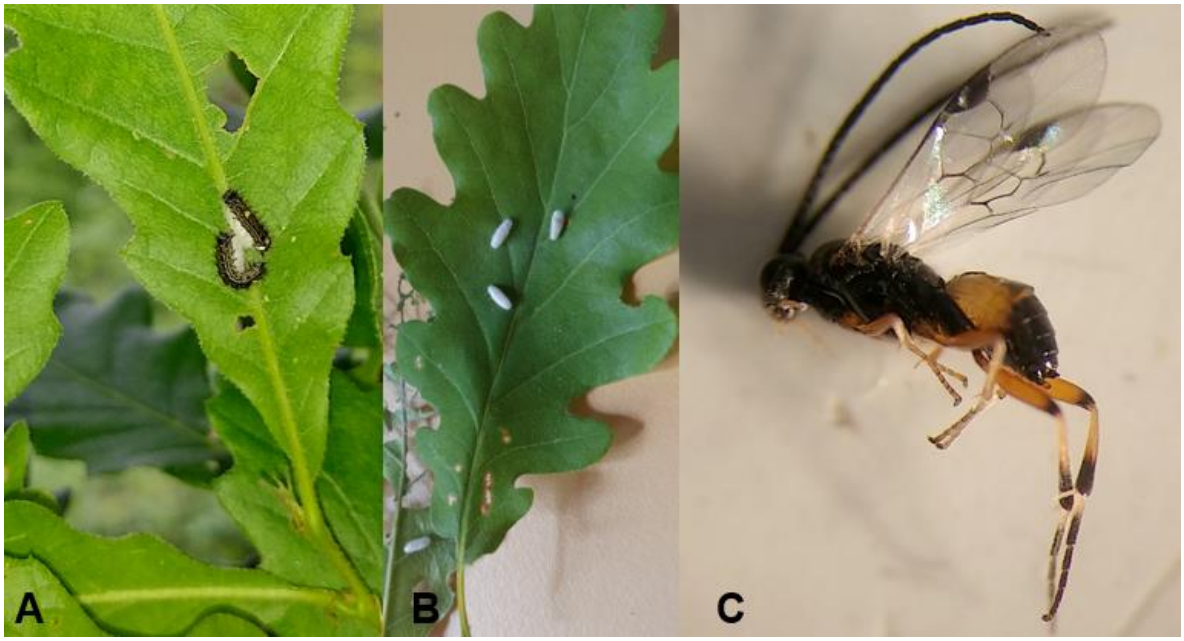

Fig. 21: *Glyptapanteles porthetriae*.
A) L3 gypsy moth larvae killed by *G. porthetriae* in the field.
B) Several cocoons of *G. porthetriae* on an oak leaf.
C) Adult female of *G. porthetriae*.

The parasitoids emerged from L2, L3 and L4 host larvae. The great majority (76 %) of host larvae died within the collection stage, 2 % moulted twice before death. Parasitoid emergence was observed between May 18th and July 1st. On average, the larvae emerged from the host 9.4 days after collection. The maximum time from the host collection to the parasitoid emergence was 36 days. Adult wasps eclosed from 54 % of the cocoons, on average eleven (5-27) days after emergence from the host larva. Fifty-eight percent of all emerged *G. porthetriae* adult wasps were males, 42 % females. Wasp eclosion was observed between May 27th and July 1st **(Fig. 22)**. Successful adult wasp eclosion correlated with the host instar at the time of collection. Wasps emerged successfully from 63 % of the cocoons when the host larvae were collected in the first two instars, but only from 39 % of the cocoons when the host larvae were collected as third or fourth instars. The difference was significant (p = 0.002). Also, the sex ratio of the wasps obtained from young and middle-aged larvae differed significantly (p = 0.024). While the sex ratio was exactly balanced in the wasps obtained from L1- and L2-collected larvae, 77 % of the wasps from L3 and L4 larvae were males. This is also reflected in the time course of wasp emergence **(Fig. 22)**.

Wasp eclosion, and especially the eclosion of female wasps from young host larvae, peaked on June 3rd **(Fig. 22)**. The highest parasitization rates from *G. porthetriae* were observed two weeks later, on June 16th. In total, 43.2 % of the *L. dispar* larvae collected on June 16th were apparently killed by *G. porthetriae*, 81.1 % of the L3 larvae and 40.0 % of the L4 larvae. These values significantly exceeded the parasitization rates of the larvae collected on the previous collection date, June 2nd. This applies to the total sample of each date (p = 0.006) as well as stage-specifically to the L3 (p = 0.002) and L4 (p = 0.001) larvae.

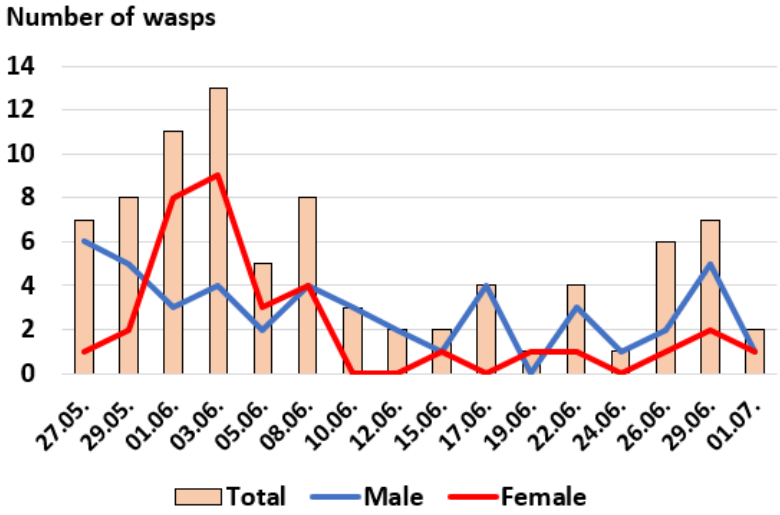

Fig. 22: Sequence of *G. porthetriae* adult wasp eclosion.

Out of five cocoons (3.1 %) identified as *G. porthetriae*, other wasp species emerged. These were identified in four cases as members of the ichneumonid subfamily Mesochorinae **(Fig. 33A)** and as one individual of the ichneumonid subfamily Cryptinae **(Fig. 33B)**. The time from pupation to wasp eclosion averaged 16 (10-21) days, which was significantly higher than for *G. porthetriae* (t-test: p = 0.036). Mesochorinae were obtained from host larvae collected in the second and third instar, Cryptinae from a third instar larva. All wasp individuals were females.

Glyptapanteles liparidis

Glyptapanteles liparidis **(Fig. 23)** was obtained from two *L. dispar* larvae. The first host larva was collected in the third instar and egressed by the parasitoid larvae in the fourth instar on June 3rd, 8 days after collection. Five larvae emerged from the host, pupated, and eclosed as adult wasps on June 12th. All individuals were females. The second host larva was collected in the fifth instar and the parasitoid larvae emerged a few hours after collection on July 16th. Twenty-five wasps emerged from this L5 host **(Fig. 23A)**. Adult wasps eclosed on July 24th

with a sex-ratio of 38:62 (female:male). The apparent mortality rate from *G. liparidis* was 0.7 % for larvae collected in the third instar and 1.6 % for larvae collected in the fifth instar.

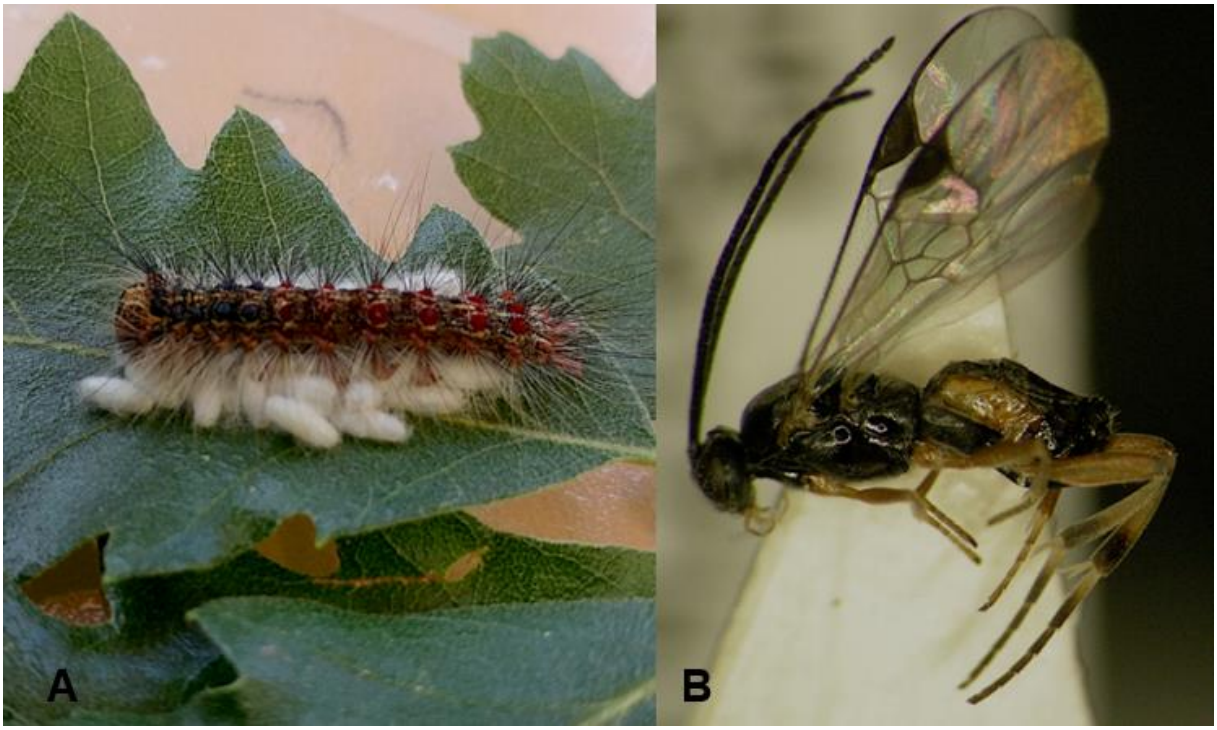

Fig. 23: *Glyptapanteles liparidis*.
A) L5 gypsy moth larva with 25 cocoons of *G. liparidis*.
B) Adult female of *G. liparidis*.

Cotesia sp.

One individual of *Cotesia* sp. – likely *C. melanoscela* – killed a third-instar host larva on June 16th, 6 days after collection. No adult wasp emerged from the yellow cocoon **(Fig. 24)**. This results in an apparent mortality rate of 0.7 % in the L3 larvae.

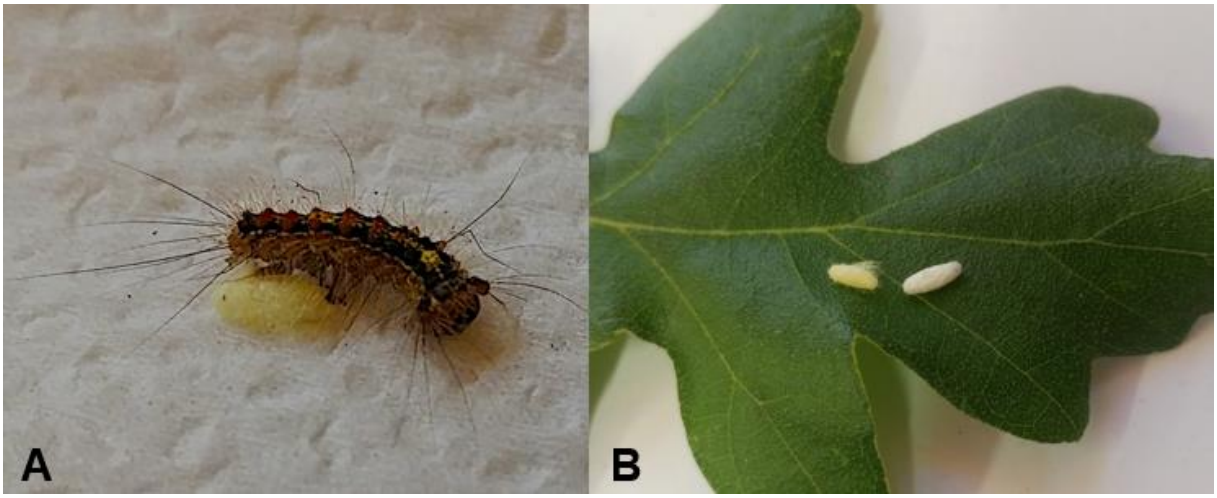

Fig. 24: *Cotesia* sp.
A) L3 gypsy moth larva with a cocoon of *Cotesia* sp.
B) Yellowish cocoon of *Cotesia* sp. (left) compared with a radiant white cocoon of *G. porthetriae* (right).

3.7.2 Ichneumonidae

Hyposoter tricoloripes

The parasitization rate from *H. tricoloripes* **(Fig. 25)** increased continuously from 1.0 % in L1-collected larvae to 8.3 % in L4-collected larvae. In the latter, the apparent morality was significantly higher than in the first two instars **(Fig. 26)**. No significant influence of the collection date on parasitization rates was detected. The peak of parasitization was observed in the L4 larvae collected on June 16th (14.3 %).

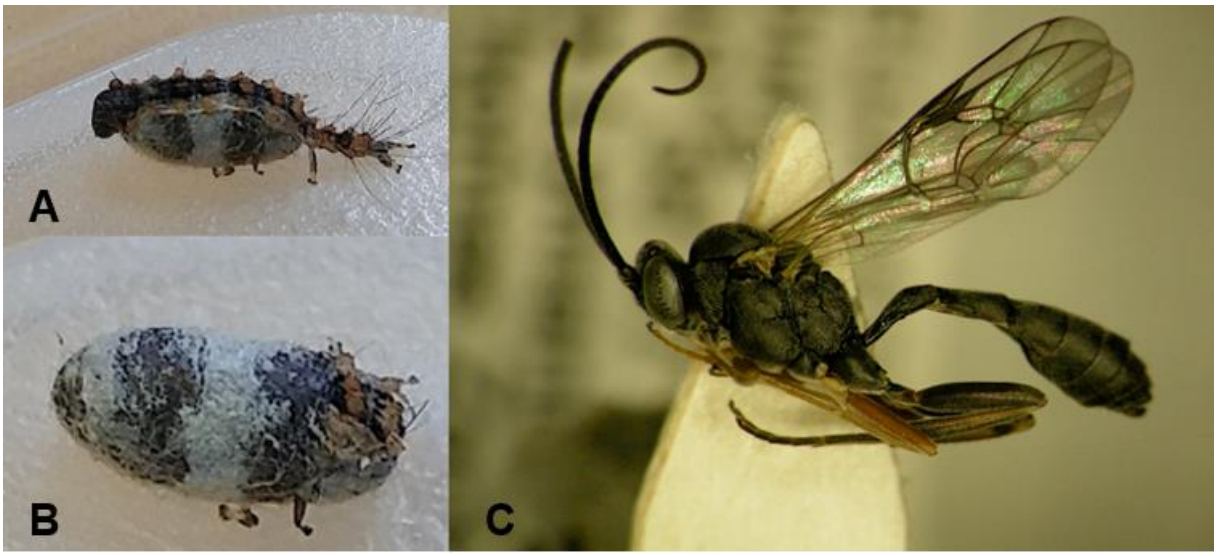

Fig. 25: *Hyposoter tricoloripes*.
A) Cocoon concealed by the skin remains of the host larva.
B) Distinctive light banded pattern of the cocoons.
C) Adult female of *H. tricoloripes*.

Hyposoter tricoloripes accounted for 4-8 % of the total parasitism in L1-L3 larvae and 18 % of the total parasitism in L4 samples. Parasitism of the final instars (L5 and L6) was not observed. All hosts were killed in the third or fourth instar, the majority (63 %) in the stage of collection. Of those that emerged from hosts collected as L3 and L4 larvae, 92 % died within the stage of collection. Host death occurred 15 (3-32) days after collection. The time to host death varied strongly between young and middle-aged larvae. In young larvae it took on average 26 days, in middle-aged larvae 9.5 days and only exceeded 10 days in 23 % of cases.

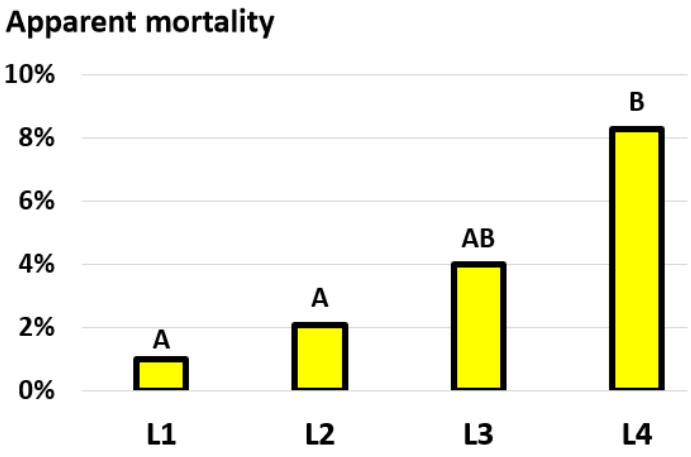

Fig. 26: Apparent mortality caused by *H. tricoloripes* based on *L. dispar* collection stages.
Letters above the columns represent statistical significances (Fisher's exact test, $p < 0.05$).

Ninety-five percent of the hosts were killed in June, one individual was killed on July 3rd. Sixty-eight percent of the parasitoid larvae successfully developed into adult wasps, with a sex ratio of 54:46 (females:males). Protandrous adult wasp eclosion occurred 9 (7-12) days after pupation, between June 12th and July 1st, with a peak on June 17th.

3.7.3 Tachinidae

Parasetigena silvestris

During collection, 12.5 % of all *L. dispar* larvae carried at least one macrotype tachinid egg on their cuticle. While tachinid eggs were rarely observed on young larvae, 34 % of all L3 larvae carried at least one egg. Subsequently, the proportion in older instars decreased continuously **(Fig. 27)**. The peak of oviposition was observed in June when 25.5 % of all larvae collected carried at least one tachinid egg, compared with 5.7 % in May, and 6.5 % in July. Stage-specific peaks were reached with 38.6 % of the L3 larvae on June 2nd and 37.1 % of the L4 larvae on June 16th. In 27 % of cases, more than one *P. silvestris* egg was observed per *L. dispar* larva (average 1.42 eggs per larva, maximum 6 eggs per larva).

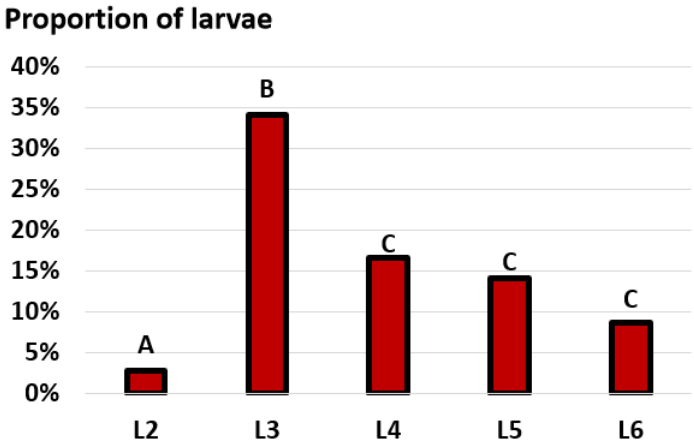

Fig. 27: Proportion of *L. dispar* larvae carrying eggs of *P. silvestris* at the time of collection.
Letters above the columns represent statistical significances (Fisher's exact test, $p < 0.05$).

The apparent mortality caused by *P. silvestris* peaked in the late instars, which showed significantly higher parasitization rates than middle-aged larvae **(Fig. 28)**. However, no significant stage-specific differences in mortality were detected in larvae collected on the same date. L4 larvae collected in early June showed a significantly higher mortality from *P. silvestris* than those collected in mid and late June ($p = 0.046$). Apart from that, no significant influence of the collection date on the stage-specific parasitization rates was observed.

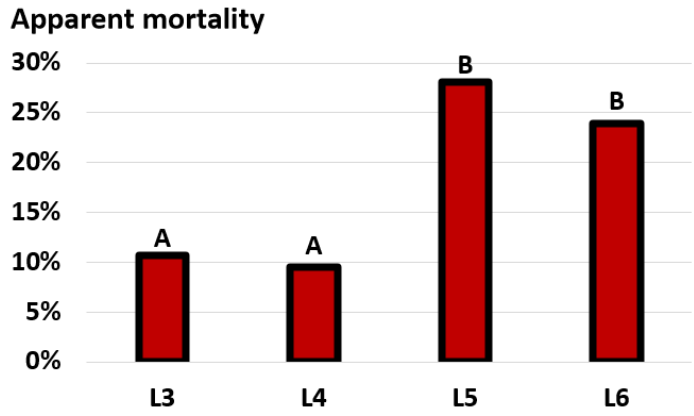

Fig. 28: Apparent mortality caused by *P. silvestris* based on *L. dispar* collection stages.
Letters above the columns represent statistical significances (Fisher's exact test, $p < 0.05$).

Larvae that were carrying *P. silvestris* eggs at the time of collection showed a higher overall mortality and total parasitization rate, as well as mortality from *P. silvestris* and *G. porthetriae*. On the other hand, larvae that carried eggs suffered less mortality from *B. pratensis*. **Table 5** gives an overview of the differences in the collection stages L3 to L6, the instars in which eggs of *P. silvestris* were frequently observed on larvae. Total pathogen mortality (16.7 % and 12.5 %; $p = 0.217$) and unknown mortality (9.0 % and 6.4 %; $p = 0.290$) were higher but not significantly increased in egg-carrying larvae. However, mortality from unknown fungi was significantly higher in L3-L6 larvae carrying *P. silvestris* eggs than in those without eggs (5.1 % and 0.7 %; $p = 0.026$).

Host death from *P. silvestris* **(Fig. 29)** occurred from the fourth to the sixth instar, mostly as L5 larvae (57 %). On average, 28 (3-83) days passed from collection to host death. However, the mean value ranged from 51 (41-83) days in L3 larvae to 10 (3-17) days in L6 larvae. In some cases, the host development was strongly retarded before death. Five *L. dispar* larvae did not moult for 30 days or more before dying. Two individuals collected as L4 larvae died in the same instar, 50 days after collection. With one exception – which died on August 24th – all host larvae died between July 3rd and 31st.

Tab. 5: Differences in mortality rates of larvae with or without *P. silvestris* eggs at the time of collection.
Green rows represent higher mortality of larvae with eggs, red rows represent the opposite.
Non-significant differences (Fisher's exact test, $p > 0.05$) are highlighted in a lighter shade.

Collection stage	Mortality factor	without eggs	with eggs	Significance level
L3+L4		n = 168	n = 65	
	Total mortality	59.2%	93.8%	< 0.001
	Total parasitization	43.5%	66.2%	0.001
	P. silvestris	6.5%	20.0%	0.004
	G. porthetriae	23.8%	36.9%	0.034
	B. pratensis	6.0%	1.5%	0.138
L5+L6		n = 97	n = 13	
	Total mortality	76.3%	84.6%	0.393
	Total parasitization	52.6%	69.2%	0.203
	P. silvestris	21.6%	61.5%	0.005
	B. pratensis	28.9%	7.7%	0.092
L3-L6		n = 265	n = 78	
	Total mortality	65.7%	92.3%	< 0.001
	Total parasitization	46.4%	66.7%	0.001
	P. silvestris	12.1%	26.9%	0.002
	B. pratensis	14.3%	2.6%	0.002

Superparasitism was observed in 11 % of *L. dispar* larvae killed by *P. silvestris*. In all cases, two maggots emerged from one host larva. All superparasitized larvae died between the fourth and the sixth instar, although 67 % died as L6 larvae. No hyperparasitism of *P. silvestris* was observed.

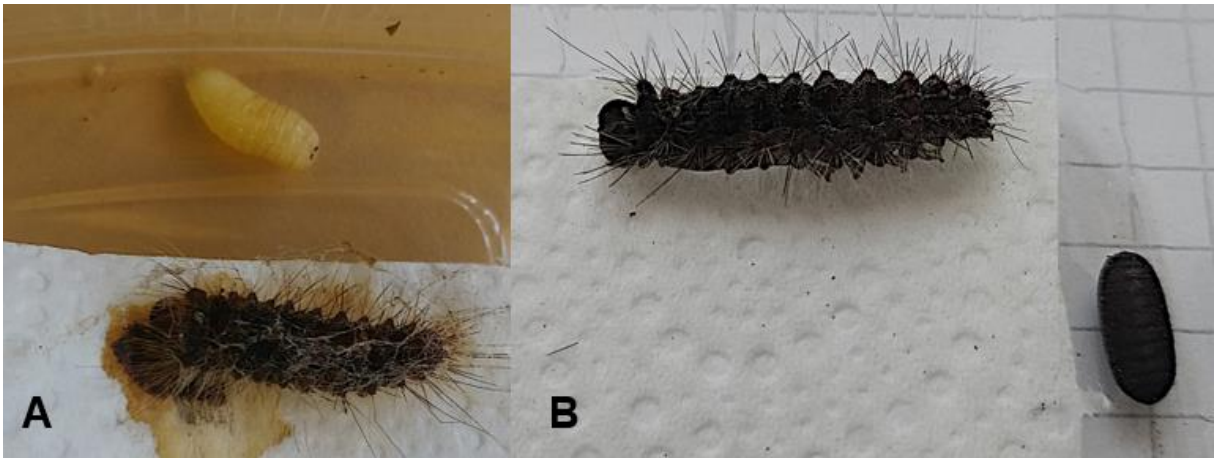

Fig. 29: *Parasetigena silvestris*.
A) Maggot of *P. silvestris* immediately after the emergence from the host larva.
B) Cadaver of the host larva and puparium of *P. silvestris* (after pupariation).

Blepharipa pratensis

In 62 dissected *Blepharipa* puparia, 29 pharate flies (47 %) were successfully identified as *B. pratensis*. In all other cases an exact determination was not possible, since 15 individuals (24 %) were hyperparasitized and 18 individuals (29 %) were damaged by fungi or adverse abiotic conditions. Hence, the presence of *B. schineri* in the sample cannot be excluded. However, *B. pratensis* was at least the dominant species of *Blepharipa* at the study site.

Apparent mortality from *B. pratensis* remained at a low but very constant level in *L. dispar* larvae collected in the first four instars. Subsequently, the parasitization rate increased significantly in L5 larvae and again from the fifth to the sixth instar **(Fig. 30)**. With 37.0 % of the L6-collected larvae, *B. pratensis* was the species that caused the highest stage-specific parasitization rate in the present study. *Blepharipa pratensis* apparently killed 16.7 % of the field-collected pupae. These are not shown in **Fig. 30** due to the small sample size of field-collected pupae (n = 12) and the associated low informative value.

Fig. 30: Apparent mortality caused by *B. pratensis* based on *L. dispar* collection stages.
Letters above the columns represent statistical significances (Fisher's exact test, $p < 0.05$).

Host death from *B. pratensis* occurred in 97 % of cases in the pupal stage **(Fig. 31)**, one host individual died as L5 and L6 larva, respectively. Up to 99 days passed between the host collection and death. The mean time ranged from 82 (74-90) days for L1-collected larvae to 17 (10-34) days for L5-collected larvae. On average, 11 (7-14) days passed from host pupation to the emergence of the maggot from the host pupa. Host death was observed from July 13th to August 21st. Sixty-four percent of the hosts were killed in the second half of July. Superparasitism was observed in 7 % of the host larvae. In all these cases, two maggots emerged from one host larva. All superparasitized host larvae were collected in the sixth instar.

Of all field-collected gypsy moth individuals that reached the pupal stage, 22 % were killed by *B. pratensis*. The value ranged from 11.8 % (L4 collections) to 70.0 % (L6 collections). Field-collected pupae did not show a significantly higher mortality from *B. pratensis* than pupae from larvae collected between the first and fourth instars and then reared in the laboratory. Pupae from individuals collected as old larvae showed significantly higher mortality from *B. pratensis* than field-collected pupae.

Fig. 31: *Blepharipa pratensis*: Puparium after the emergence of the maggot from a gypsy moth pupa.

In the dissections of *B. pratensis* puparia, which were obtained from field-collected hosts, hyperparasitization was found in 24.2 % of the puparia. The living adult hyperparasitoids were identified as the chalcid wasps *Perilampus ruficornis* (Fabricius) and *Perilampus aeneus* (Rossius), with *P. ruficornis* predominating **(Fig. 32)**. Puparia obtained from hosts collected as middle-aged larvae (L3 and L4) were most frequently hyperparasitized (54.5 %). This ratio was significantly higher than in young larvae (6.3 %, $p < 0.001$). Puparia from hosts collected as late instars (L5 and L6) showed a hyperparasitization rate of 24.2 %, which was neither significantly different from young larvae ($p = 0.127$), nor from middle-aged larvae ($p = 0.070$). The sex ratio of the *Perilampus* individuals was 43:57 (females:males).

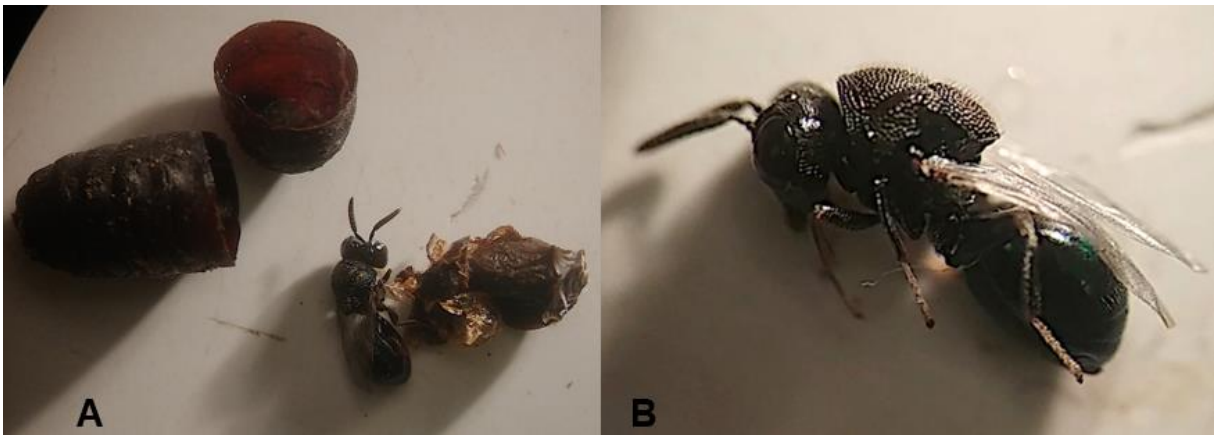

Fig. 32: *Perilampus* sp.
A) Dissected puparium of *B. pratensis* with adult *Perilampus* wasp and remains of the fly cadaver.
B) Adult *Perilampus ruficornis* wasp.

3.7.4 Hyperparasitism of field-collected *G. porthetriae* cocoons

With 57.8 %, the eclosion rate of field-collected *Glyptapanteles porthetriae* cocoons was very similar to cocoons obtained from field-collected *L. dispar* larvae. However, *G. porthetriae* adult wasps only emerged from 13.3 % of the field-collected cocoons, while hyperparasitic wasp species emerged from 44.4 % of the field-collected *G. porthetriae* cocoons. In other words, 76.9 % of all adult wasps that emerged from field-collected *G. porthetriae* cocoons were hyperparasitoids. While adult eclosion of *G. porthetriae* wasps was observed six (1-15) days after cocoon collection, the emergence of hyperparasitoid wasps was observed on average after 29 (13-34) days.

The 20 hyperparasitoid wasps obtained from *G. porthetriae* cocoons belonged to at least five species of three hymenopteran families. Members of the chalcidid family Pteromalidae **(Fig. 34D)** emerged from ten cocoons (22.2 %), several ichneumonid wasps of the subfamily Cryptinae **(Fig. 34A-C)** (1x *Gelis* sp.; five individuals of two different but unidentified species) from six cocoons (13.3 %) and *Eurytoma* sp. (Chalcidoidea, Eurytomidae) **(Fig. 34E-F)** from four cocoons (8.9 %).

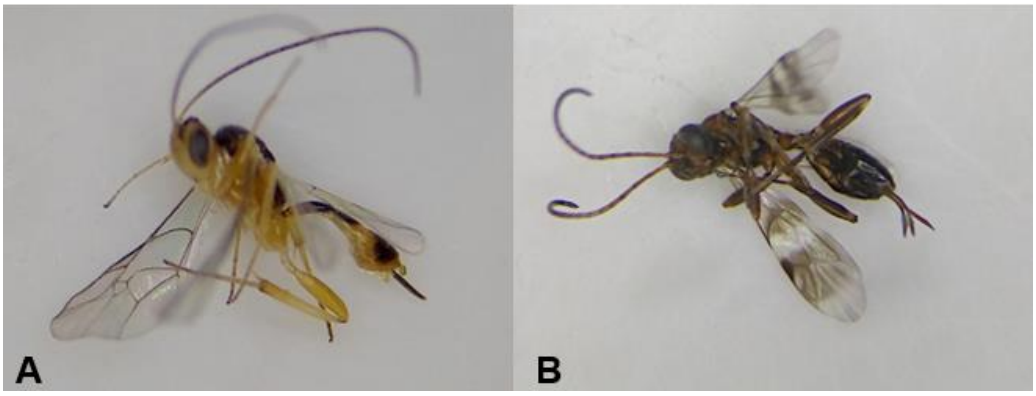

Fig. 33: Hyperparasitoids, emerged from *G. porthetriae* cocoons obtained from field-collected *L. dispar* larvae. Determined as:
A) Subfamily Mesochorinae (Ichneumonoidea, Ichneumonidae).
B) Subfamily Cryptinae (Ichneumonoidea, Ichneumonidae).

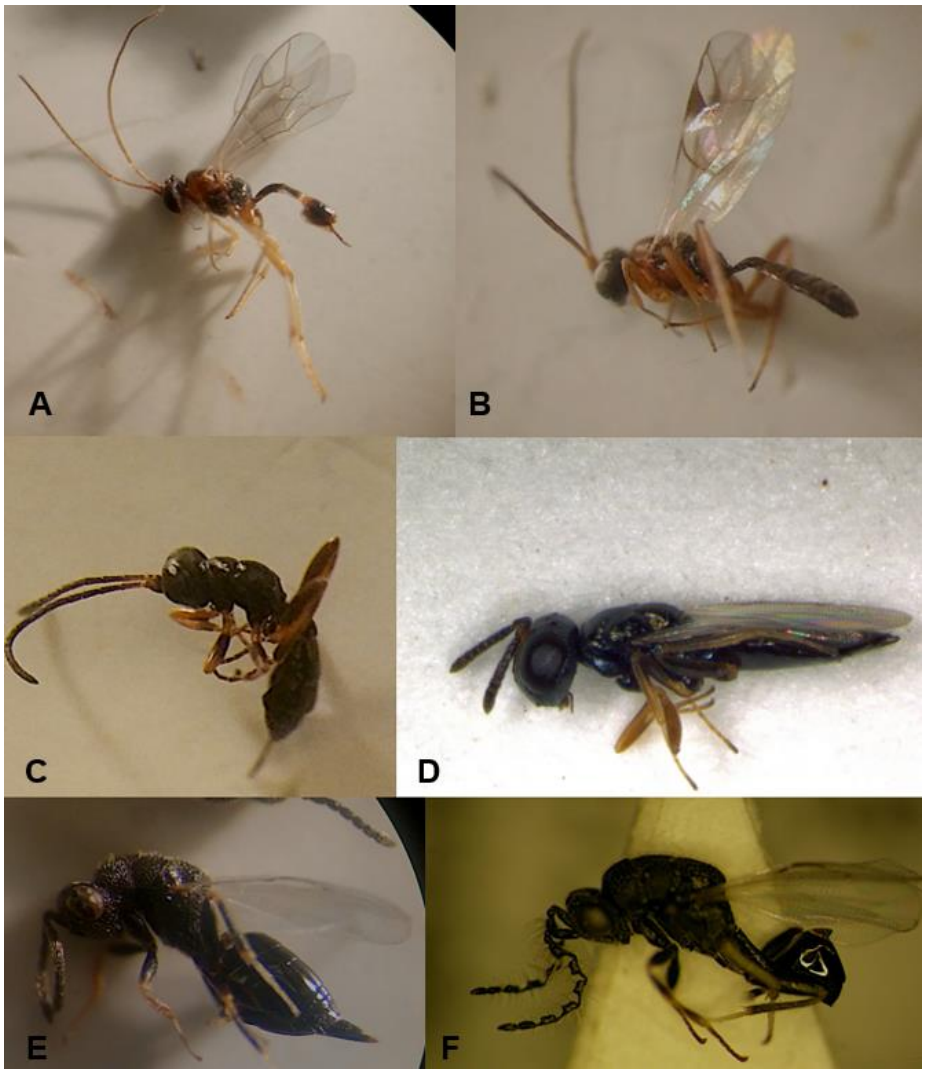

Fig. 34: Hyperparasitoids, emerged from field-collected *G. porthetriae* cocoons (Pseudohyperparasitoids). Determined as:
A-C) Subfamily Cryptinae (Ichneumonoidea, Ichneumonidae).
D) Family Pteromalidae (Chalcidoidea).
E-F) Family Eurytomidae (Chalcidoidea).

3.8 Pathogens of *L. dispar* larvae

Entomopathogens appeared to be responsible for 23 % of all larval mortality. Depending on the development stage at the time of collection, the apparent pathogen mortality ranged from 10.7 % (L3) to 41.4 % (L1) **(Tab. 4)**.

3.8.1 *L. dispar* multinucleocapsid nuclear polyhedrosis virus (NPV)

NPV **(Fig. 40A)** was clearly the dominant mortality agent among the pathogens. NPV was apparently involved in 16.7 % of the total mortality in this study. At the stage-specific level, the ratio ranged from 8 % (L6) to 48 % (L1) of total mortality. Among the pathogens, NPV was involved in 73.0 % of all cases and caused 43 % (L6) to 88 % (L2) of the pathogen mortality. Mixed infections were rarely observed. Mixed infection by NPV and an unidentified fungus was observed in two first instar larvae. Two other individuals were infected with NPV and *E. maimaiga*.

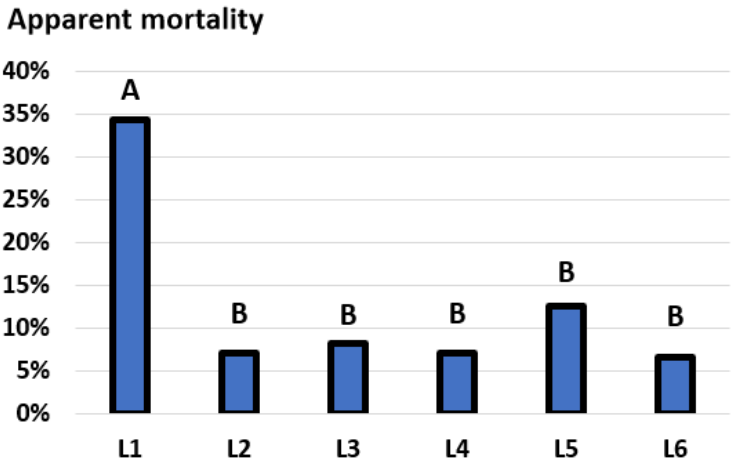

Fig. 35: Apparent mortality caused by NPV based on *L. dispar* collection stages.
Letters above the columns represent statistical significances (Fisher's exact test, $p < 0.05$).

Mortality from NPV was significantly higher ($p < 0.001$) in larvae collected as first instars than in larvae collected in any other instar. From second instar, a relatively constant mortality of 6.5 % (L6) to 12.5 % (L5) without significant stage-specific differences was observed **(Fig. 35)**. No significant influence of the collection date on the stage specific NPV mortality rates was observed.

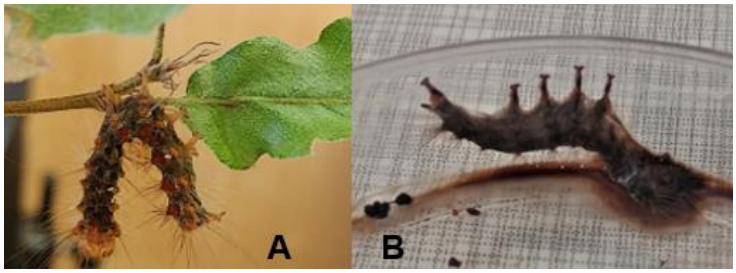

Fig. 36: NPV – macroscopic symptoms.
A) Dead gypsy moth larva characteristically attached to a twig in inverted "V"-shape.
B) Liquefaction of NPV infected cadaver.

Death occurred on average 20.7 (1-83) days after collection. Only 44 % of the larvae killed by NPV died within the first two weeks after collection. For L1-collected larvae, a bimodal pattern in the sequence of mortality by NPV was observed **(Fig. 37)**, with peak mortality four and 25 days after collection, respectively. For L2 larvae, the pattern was unimodal with a peak 25 days after collection. For all other collection stages, the sequence of mortality from NPV did not follow a clear pattern.

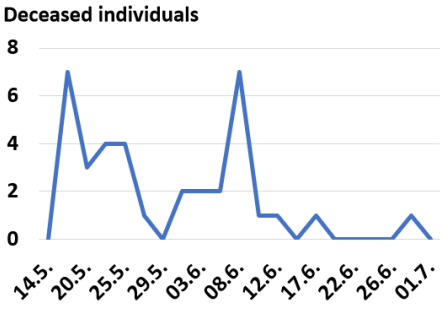

Fig. 37: Number of deceased *L. dispar* individuals per day in larvae collected as first instars on May 14th, 2020.

Ten percent of all larvae collected passed through two or three instars before death. In 56 % of cases, the host larvae died within the stage of collection and 24 % of these larvae remained in the instar of collection for 20 days or more before they died. In one case, a third instar larva died within the collection stage after 57 days.

3.8.2 *Entomophaga maimaiga*

Entomophaga maimaiga **(Fig. 39)** was apparently involved in 2.0 % of the total mortality and 8.7 % of the pathogen mortality in this study. While *E. maimaiga* was not observed in larvae collected in the first three instars, it caused 7.2 % of the total mortality and 33.3 % of the pathogen mortality in larvae collected between the fourth and sixth instar. The highest mortality rate was observed in L5 larvae with 4.7 % **(Fig. 38)**.

Mortality from *E. maimaiga* was observed between June 10th and July 20th. Most of the larvae were collected and died in the second half of June. With 6.1 %, larvae collected in the second half of June showed a significantly higher mortality rate ($p = 0.024$) from *E. maimaiga* than larvae collected on June 2nd (0.8 %).

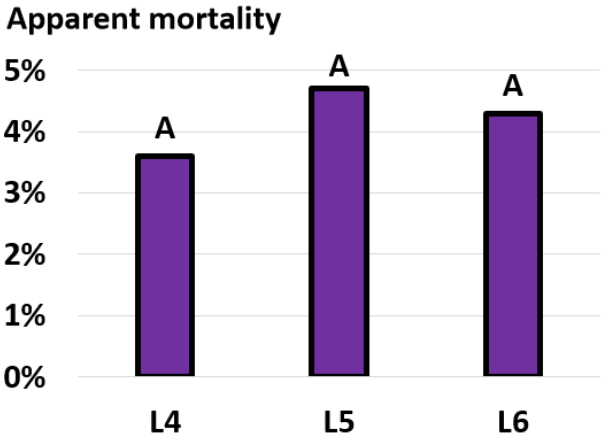

Fig. 38: Apparent mortality caused by *E. maimaiga* based on *L. dispar* collection stages.
Letters above the columns represent statistical significances (Fisher's exact test, $p < 0.05$).

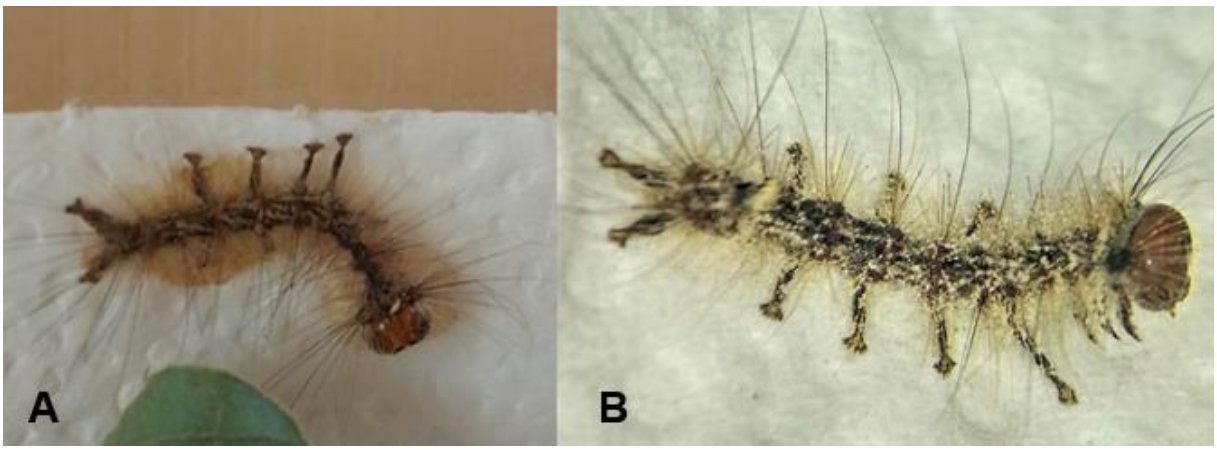

Fig. 39: *Entomophaga maimaiga* – Macroscopic symptoms.
A) Characteristic extension of the abdominal legs from the body at an angle of 90°.
B) Intensive formation of external conidia on a host cadaver.

Host death occurred fast and within the stage of collection, also in larvae that were co-infected with *E. maimaiga* and NPV. On average, the host died four (1-8) days after collection. In 30 % of the host cadavers only conidia were found on microscopic examination. All were collected as L4 larvae and died between June 10th and June 19th. In the cadavers of all host larvae collected after June 23rd, conidia and azygospores were detected **(Fig. 40B)**.

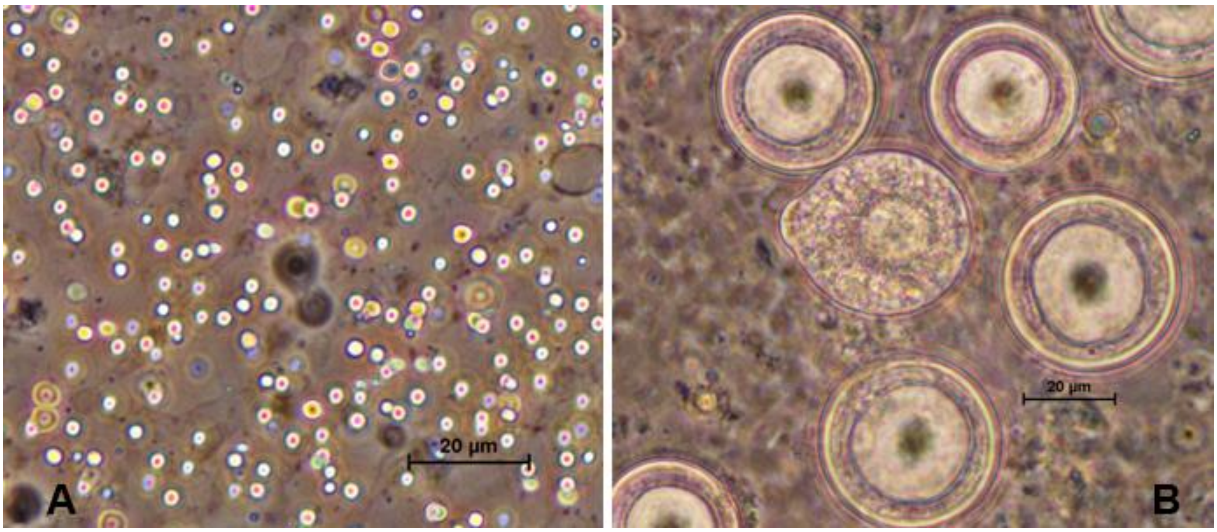

Fig. 40: Detection of pathogen infections with phase contrast microscopy.
A) Occlusion bodies of NPV.
B) Pear-shaped conidia and thick walled azygospores of *Entomophaga maimaiga*.

3.8.3 *Endoreticulatus schubergi*

One *L. dispar* individual collected in the sixth instar on July 16th died on July 29th in the prepupal stage apparently due to the microsporidium *E. schubergi*. This corresponds to 14 % of pathogen mortality of L6 larvae.

3.8.4 Unknown fungi

Non-identified fungi were apparently involved in 4.8 % of the total mortality and 20.9 % of the pathogen mortality. Depending on the collection stage, the values ranged from 0 % (L5) to 9 % (L1) of the total mortality and 0 % (L5) to 39 % (L2) of the pathogen mortality. The highest apparent mortality rates appeared in the first three instars of *L. dispar*; however, the mortality rates did not differ significantly between any collection stages **(Fig. 41)**.

Fig. 41: Apparent mortality caused by unknown fungi based on *L. dispar* collection stages.
Letters above the columns represent statistical significances (Fisher's exact test, $p < 0.05$).

Seventy-seven percent of the host larvae died within the instar of collection, another 18 % in the subsequent stage. On average, death occurred 18 (6-43) days after collection. Fifty percent of the hosts died within 14 days after collection.

3.9 Unknown larval and pupal mortality

In 18 % of the deceased larvae and 60 % of the field-collected pupae, the cause of death remained unclear. Depending on the stage, the cause of death could not be determined in 2 % (L5) to 26 % (L2) of the individuals. Unknown mortality was highest in young larvae **(Fig. 42)**. Fifty-seven percent of the larvae died within the stage of collection, 36 % in the subsequent stage, and 6 % passed through 3 or more instars. Death occurred on average 20 (0-99) days after collection. Fifty percent of the larvae died within 14 days.

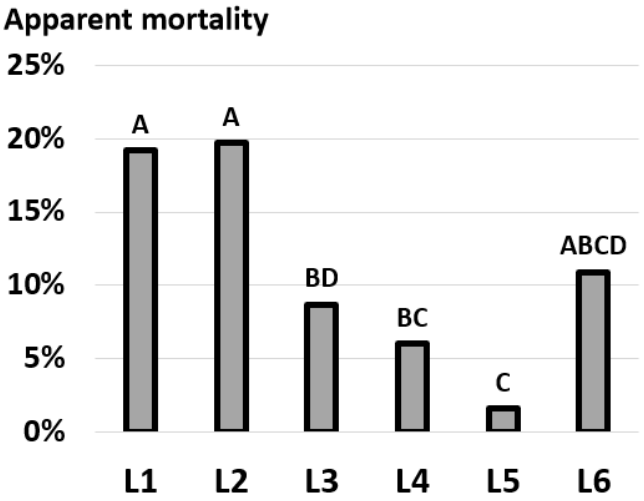

Fig. 42: Apparent mortality by unknown causes based on *L. dispar* collection stages.
Letters above the columns represent statistical significances (Fisher's exact test, $p < 0.05$).

3.10 Marginal attack rates and *k*-values

3.10.1 Stage-specific *k*-values

Figure 43 shows the *k*-values, calculated specifically for each stage of death according to **Equation 6**. The *k*-values fluctuate within a narrow range between 0.273 and 0.311 for eggs, pupae, and the larval instars L2, L4, L5, and L6. In L3 larvae with 0.215 and particularly in L1 larvae with 0.143, the *k*-values were markedly lower.
Parasitism was the most important mortality factor and accounted for more than half of the stage-specific *k*-value in all stages except of eggs and L1 larvae. Since no host larva was killed by parasitoids in the first instar, a *k*-value of 0.000 results in this stage. In other stages, the effects of parasitoids ranged from 0.112 (eggs) to 0.270 (pupae).

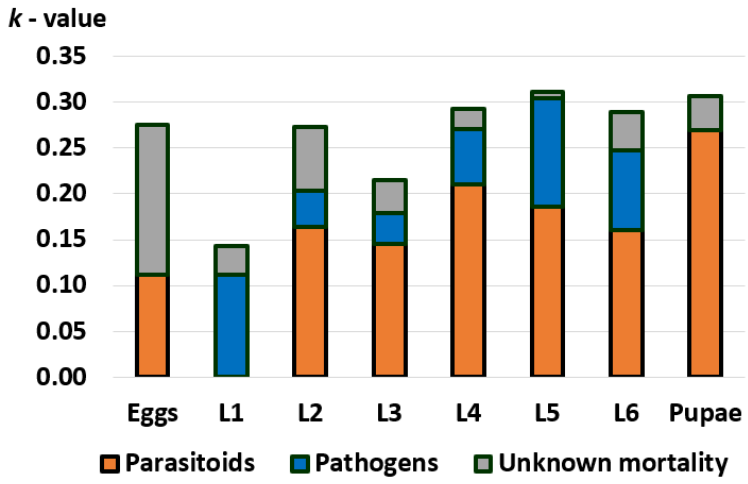

Fig. 43: Killing power and relative importance of parasitoids, pathogens and unknown causes as mortality agents observed in different *L. dispar* development stages.

No pathogen-induced mortality was found in *L. dispar* eggs or pupae. In larvae, the influence of pathogens ranged from 0.034 (L3) to 0.112 (L1). Unknown mortality was the dominant mortality factor of gypsy moth eggs ($k = 0.163$), but played a much smaller role in larvae, where the values ranged from 0.007 (L5) to 0.069 (L2).

3.10.2 Factor-specific *k*-values

Figure 44 shows *k*-values **(Equation 9)** for individual mortality factors during the entire development of *L. dispar*, from the egg to the adult moth.

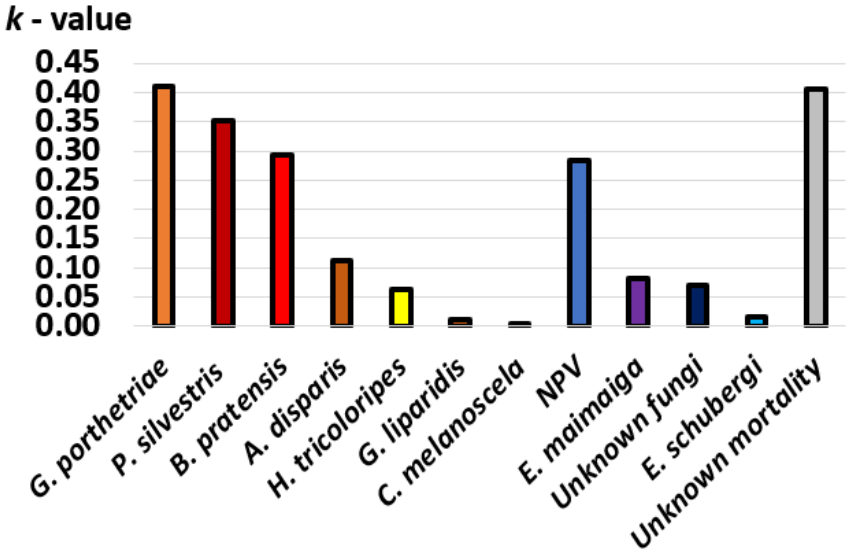

Fig. 44: Killing power of individual mortality factors from egg hatching to moth eclosion of *L. dispar*.

The egg parasitoid *A. disparis* achieved a *k*-value of 0.112. The braconid wasp *G. porthetriae* had the highest killing power, with a *k*-value of 0.410 and was closely followed by unknown mortality factors (*k* = 0.406). The three most abundant parasitoid species, *G. porthetriae* and the tachinids *P. silvestris* and *B. pratensis* together accounted for 50 % of the total killing power, while the eight remaining identified mortality factors combined accounted for 30 %.

With 0.284, the *k*-value of NPV – the most effective pathogen – was just below the value of the third most important parasitoid (*B. pratensis*). The three least important parasitoid species (*H. tricoloripes*, *G. liparidis*, *C. melanoscela*) and pathogens (*E. maimaiga*, unknown fungi, *E. schubergi*) together accounted for 11.8 % of the total generational killing power. Among pathogens, the *k*-value for *E. maimaiga* (0.081) was slightly higher than the value for unknown fungi (0.071).

3.11 Sex-ratio of *Lymantria dispar*

The sex ratio of the adult gypsy moths that emerged from field-collected larvae and pupae was 31:69 (females:males). However, the sex-ratio differed significantly (χ^2-test: $p < 0.001$) depending on the collection stage. The sex-ratio of the adult moths was highly male-biased (73:27) in individuals collected as young larvae and moderately male-biased (62:38) in individuals collected as middle-aged larvae. A strongly contrasting pattern was observed in moths from individuals collected as old larvae. While all 19 moths that emerged from larvae collected in the fifth instar were males, all six individuals obtained from L6 larvae were females. The sex ratio of adults obtained from field-collected pupae was roughly balanced **(Fig. 45)**.

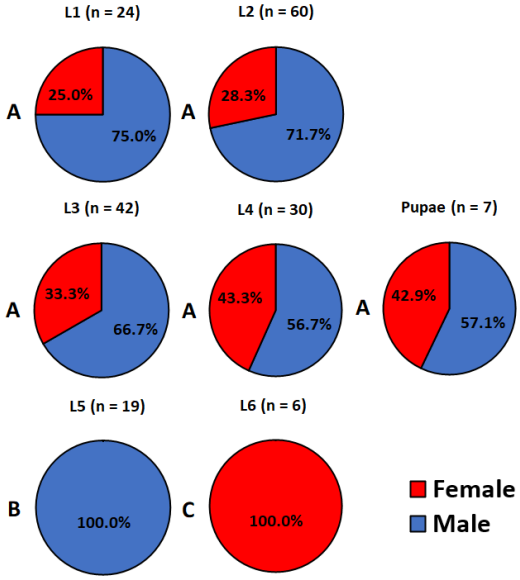

Fig. 45: Sex-ratio of adult *L. dispar* moths based on collection stages. Letters on the left of circles represent statistical significances (Fisher's exact test, $p < 0.05$).

3.12 Within-generation variability in population size

During the 2020 vegetation period, there was a strong decrease in population density, which became evident in the number of *L. dispar* individuals collected from June 30th to July 30th **(Fig. 46)**. During this period, a standardized and reproducible sampling was carried out (burlap bands). Thus, a conclusion on the population density can be made for this period, in contrast to the collection dates earlier in the season, where these requirements were not met. From July, the population was very sparse and despite an intensive search hardly any individual was found outside the burlap bands. In the semi-field rearing, pupation started on June 29th, but only twelve pupae were on four collection days in the field in July. In the second half of July, adult individuals of *Calosoma sycophanta* (Coleoptera, Carabidae), a larval and pupal predator of the gypsy moth, were observed more frequently under the burlap bands than larvae or pupae of *L. dispar*. In addition, not a single adult *L. dispar* individual was observed in the field.

This impression of a decreasing population density was also confirmed by observations of the leaf damage and an inspection of the study site in spring 2021. In contrast to the total defoliation on the study site in 2018 and 2019, hardly any leaf damage was observed in the late spring and early summer of 2020. In spring 2021, virtually no fresh and intact egg masses were observed, indicating that the population has completely collapsed and reached the latency period again.

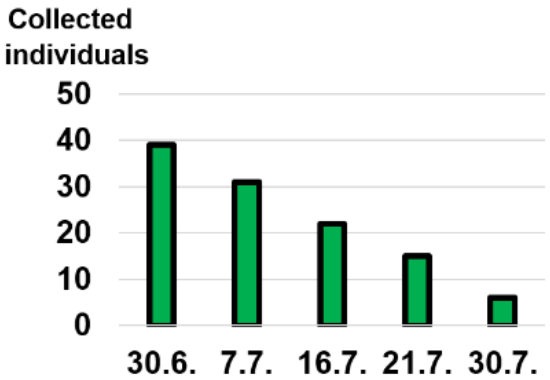

Fig. 46: Number of *L. dispar* individuals collected from 72 trees equipped with burlap bands from June 30th to July 30th.

4 Discussion

4.1 Population density, phenology of *L. dispar*, and weather conditions

4.1.1 Population density and phenology of *L. dispar* in 2020

Population density in spring

With 13.0 egg masses per tree, the egg mass density at the study site was at a high level compared to the results of previous Austrian studies. KALBACHER (2008) observed egg mass densities between 9.7 (2004) and 13.3 (2005) egg masses per tree, HOCH (1995) values from 0.0 to 4.8. However, egg mass density can also reach markedly higher values, for example in Slovakia, in 1992 densities of up to 30.9 egg masses per tree were observed (ZÚBRIK and NOVOTNÝ, 1997).
On the other hand, the number of eggs per egg mass was at a low level with a median of 192 eggs. Only two egg masses exceeded an egg number of 300 with 401 and 640 eggs, respectively. The latter value is a clear outlier **(Fig. 9)**, possibly in this case two adjacent egg masses were mixed up unintentionally.

KALBACHER (2008) observed a very similar population density (13.3 egg masses, 200 eggs) in spring 2005, the year prior to the collapse of the studied gypsy moth population. A high number of egg masses per tree combined with a low number of eggs per egg mass is characteristic for the retrogradation period of the population cycle of *L. dispar*. The male-biased sex ratio and high egg mortality rate are also characteristic for this period (WELLENSTEIN and SCHWENKE, 1978). These changes are primarily driven by two factors. Firstly, the defoliation of the study site in the previous years probably correlated both on a quantitative and a qualitative level with restricted food supply. Defoliation is associated with increasing concentrations of phenolic compounds in the leaves. An impaired food supply results in decreased pupal weights of the females, which correlates directly with reduced fecundity (ROSSITER et al., 1988). On the other hand, the prevalence of entomopathogens generally increases with the host density (HOCH et al., 2001). It has been shown that NPV (IL'INYKH et al., 2009; AKHANAEV et al., 2020) and microsporidia (McMANUS and SOLTER, 2003; GOERTZ, 2004) reduce the fecundity in sub-lethally infected females.

Thirteen egg masses of 222 eggs correspond to 2,886 eggs per tree. Without the influence of regulating factors, this number would probably have been sufficient to defoliate the forest at the study site in 2020. WELLENSTEIN and SCHWENKE (1978) state critical egg numbers of 1,400 eggs as sufficient to defoliate a 50-year-old oak and 490 eggs for a 20-year-old oak, during retrogradation period. Most of the trees at the study site were estimated to be less than 50 years old. Furthermore, egg masses were only counted up to a height of four metres, but not in higher parts of the crown. In 1993, HOCH et al. (2001) observed defoliation of most trees in Burgenland after a spring population density of 2,563 eggs per tree.

Phenology of L. dispar

Figure 47 relates the phenology of *L. dispar* at the study site to the phenological development observed in Burgenland in the years 1993-1995 (SCHOPF and HOCH, 1997) and 2003-2004 (KALBACHER, 2008). The high temperature in early spring resulted in a rather early hatching in 2020. Subsequently, the low temperature in May strongly delayed the development of the young larvae. While in the present study L1 larvae were observed over a period of seven weeks and L2 larvae over six weeks, the observation period in the five comparative years of the previous studies was 4.4 (L1) and 3.4 (L2) weeks on average. As a result, both L1 and L2

larvae were observed later in the season in 2020 than in any of the five comparison years. With the increase in temperature in June 2020 **(Fig. 11)**, larval development also accelerated markedly and the instars L3-L6 were passed rapidly. However, the developmental delay of the young larvae could no longer be fully made up, resulting in a late onset of pupation and adult eclosion.

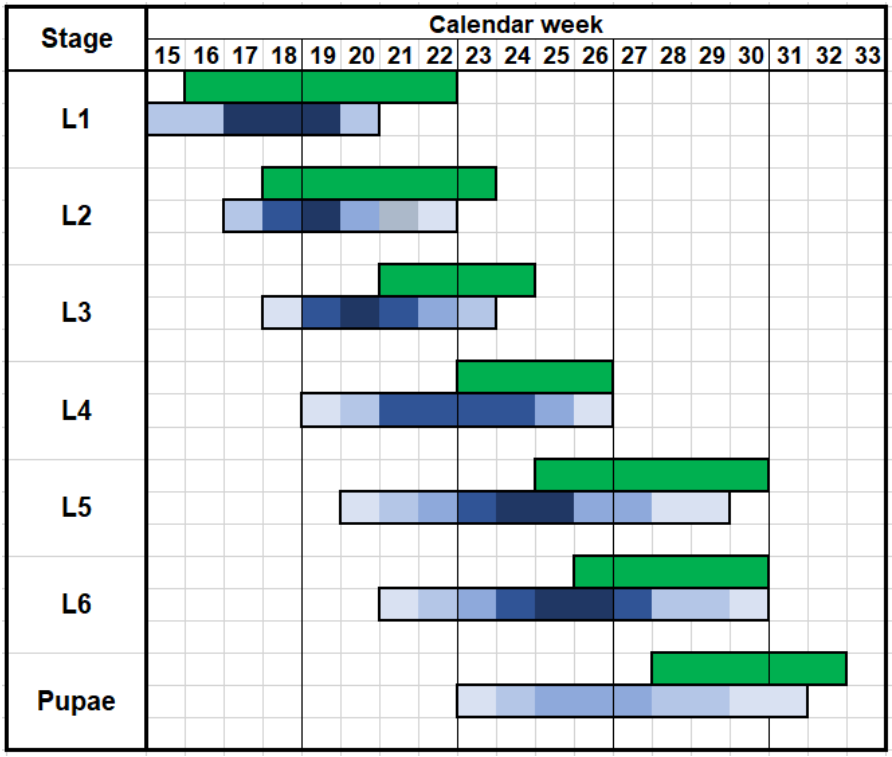

Fig. 47: Phenology of *L. dispar* in Eggenburg 2020 (green) compared to preceding observations (blue) in Burgenland in 1993-1995 (SCHOPF and HOCH, 1997) and 2003-2004 (KALBACHER, 2008).
Overlaps in preceding studies are considered by the brightness of the blue bars: the darker the shade of blue, the more overlaps.

4.1.2 Weather conditions

Figure 48 gives an overview of climate data and weather conditions for the years of the gypsy moth population outbreak in the study area. The outbreak was triggered in 2018 with unusually high temperatures in April and May and low precipitations in early spring. With 15.0 °C in April and 18.2 °C in May, the mean monthly temperatures were 5.0 °C (April) and 3.3 °C (May) above the long-term average and 2.5 °C (April) and 1.6 °C (May) warmer than in any other year of the past decade (ZAMG, 2020a). April 2018 was the warmest April recorded in Austria since 1800 and May the warmest since 1868 (ZAMG, 2018b). This supports the previous knowledge about the important influence of warm and dry spring weather in the initiation of population outbreaks of *L. dispar* (WELLENSTEIN and SCHWENKE, 1978, HOCH et al., 2001). However, spring drought is more likely to relate to early spring and the above-average precipitation in May 2018 was likely an important beneficial factor in triggering the outbreak. ZÚBRIK et al. (2021) highlighted the role of balanced weather in May for the initiation of outbreaks in Slovakia in the recent decades. It was shown that water stress of host plants impairs the food conversion of *L. dispar* larvae. Although this is compensated for by increased food uptake by individual larvae (CASTAGNEYROL et al., 2018), it impairs the overall energy yield at the population level and thus, also the potential for the populational fecundity.

February and March 2018 were unusually cold and frosty (ZAMG, 2018a). While heavy late frost in spring impairs gypsy moth population dynamics adversely (ZÚBRIK et al., 2021), cold periods in February and March may also have advantages for initiating the population outbreak. The vegetation phenology was delayed by 2-3 weeks compared to the long-term average (ZAMG, 2018a). Consequently, *L. dispar* egg larvae were also undoubtedly far away from terminating winter diapause and moderate frosts did not pose a serious risk (MADRID and STEWART, 1981). The delay in phenology has probably shifted the gypsy moth hatching to a later period of the exceptionally warm April. This presumably provided outstanding high field temperatures during the development of young larvae, resulting in a very rapid larval development. This mitigates the temporal exposure of the larvae to natural enemies.

In 2018, gypsy moth population outbreaks also started in Germany (LEMME et al., 2019) and Slovakia (ZÚBRIK et al., 2021). In the Czech Republic local defoliation was already observed in 2017, but gradations became more widespread and more intense in 2018 (HOLUŠA et al., 2020). This shows once again the high temporal synchronization in the population dynamics of *L. dispar* across large regions.

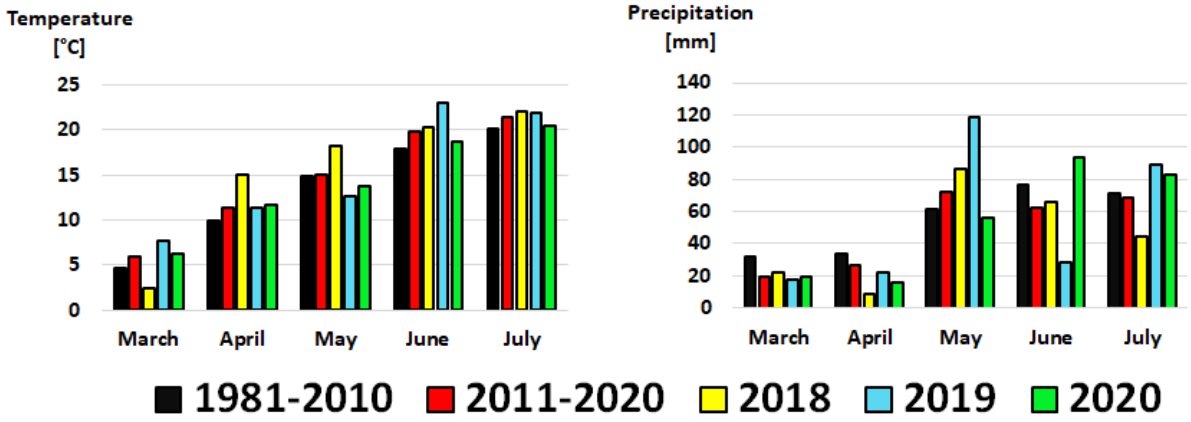

Fig. 48: Mean monthly temperature and monthly precipitation for the periods 1981-2010, 2011-2020 and the years 2018, 2019 and 2020.
Data represent an average value for the ZAMG stations in Krems and Retz in order to mitigate local fluctuations (Source: ZAMG, 2020a).

4.2 Hatching of *L. dispar* eggs and egg mortality

The high egg mortality from parasitoids and unknown factors may have contributed significantly to the fact that the results of the present study differ markedly from previous results observed at similar population densities in Central Europe. The significant reduction of the population prior to the development of *L. dispar* larvae de facto corresponds to a reduction by half of the population density and thus, the number of potential hosts for natural enemies of *L. dispar* larvae **(Fig. 60)**.

4.2.1 Unknown egg mortality

Field-collected egg masses showed an unusually high proportion of eggs with unhatched embryos (27.7 %) **(Fig. 15)**, which has not yet been reported for *L. dispar* in Central Europe previously. WELLENSTEIN and SCHWENKE (1978) describe increased egg mortality rates of > 10 % in retrogradation periods compared to 1-10 % during progradation. HOCH (1995) reported egg mortality rates between 0.2 % and 12.4 % in two populations over a period of two

years, without any presence of egg parasitoids. ZÚBRIK and NOVOTNÝ (1997) observed the egg mortality at several Slovakian locations in four years in the 1990s and reported an average value of 3.6 % (0.3 % - 13.3 %) egg mortality caused by unknown factors. In 1993 and 1995, when the populations were declining at most study sites, the unknown egg mortality averaged 7.8 % and 8.8 %, respectively. WERMELINGER (1995) reported egg mortality rates of 8.0% to 10.6 % during retrogradation in Switzerland.
In Russia, for example, ILYINYKH et al. (2017) observed rates of 70 % egg mortality in a population during retrogradation, compared to 15 % during progradation. They assumed embryonic mortality as the main factor causing the population collapse. Embryonic mortality may be caused by various factors.

Impaired food supply at high population densities can lead to increased embryonic mortality. The overall nutritional composition of *L. dispar* eggs during retrogradation is similar to other periods of the population cycle; however, significantly lower levels of specific key proteins of embryogenesis were observed during retrogradation (ILYINYKH et al., 2017).

The occurrence of two egg masses without any larval or parasitoid emergence in the present study indicates the involvement of abiotic factors. One possible explanation is the cold spell at the end of March and the first week of April 2020 with unusually heavy frost in the study area, less than a week prior to egg hatching. *Lymantria dispar* eggs have a high frost tolerance during winter diapause and the supercooling point (SCP) of eggs only increases slightly before the hatching in spring (MADRID and STEWART, 1981). However, it was shown that cold hardiness is not exclusively regulated by the SCP, mortality occurs at temperatures above the SCP, and eggs with the same SCP can have different cold hardiness (SULLIVAN and WALLACE, 1972). Unfortunately, intensive research on cold hardiness of gypsy moth eggs has focussed on very low temperatures during winter diapause, while data to the effects of early spring frosts on post-diapausing eggs are very rare. After a late frost of -2 °C in former Yugoslavia, a mortality of 74 % of the “egg larvae” is reported (WELLENSTEIN and SCHWENKE, 1978). However, it is unclear if this refers to eggs or newly hatched larvae.

Another abiotic factor might be unfavourable storage conditions after the collection. However, since most larvae hatched within the first week of collection, this seems to be an unlikely reason. Since the emergence of egg parasitoids began much later than the hatching of *L. dispar* eggs, parasitized eggs were exposed longer to the storage conditions. Thus, egg mortality caused by this factor would probably have mainly affected parasitized eggs, shifting mortality from parasitization to unknown mortality rather than reducing the larval hatching rate. Random dissections of several unhatched eggs showed that they contained predominantly pharate adult wasps in some and lepidopteran embryos in other egg masses. In the egg masses without hatching, no wasps were found in unhatched eggs.

Sublethal infections by microsporidia have been shown to cause reduced *L. dispar* hatching rates (GOERTZ, 2004; GOERTZ and HOCH, 2008b). In the case of *Nosema* sp., a proportion of the eggs – particularly those that were last oviposited by the female – typically desiccate and the embryo dies (WEISER, 1998). It was also shown that NPV causes significantly increased egg mortality after sublethal infections (AKHANAEV et al., 2020). Reduced fertility was reported in all cases but was not observed in the present study. However, not all authors clearly differentiated between fertility (the proportion of fertilized and viable eggs) and natality (the hatching success of fertilized eggs) and sometimes used the terms synonymously.

Another explanation might be predatory activity. BATHON (1993) names the predatory mites *Allothrombium wolfii* and *Thrombidium holosericeum* (Acari, Thrombidiidae) as the most important arthropod egg predators of *L. dispar* in Central Europe. In the present study, mites were also observed at sucking on eggs, however, in low prevalence. The mites were not precisely identified, but representants of at least two species were observed, which differed clearly in their morphological appearance from the two species mentioned **(Fig. 49)**. *Allothrombium* sp. can only feed on eggs after they have been uncovered from abdominal hair by other factors (CAMERINI, 2009) and leaves crumpled remains of host eggs (SAEIDI, 2011). Such remains were rarely observed, so mites are not assumed as a major factor in the unknown egg mortality in this study.

Fig. 49: Predatory mites, observed to suck on *L. dispar* eggs.

Parasitoids could have contributed to embryonic mortality in two ways. First, females of *A. disparis* are known to host feed on *L. dispar* eggs (PARKER, 1933). The literature, however, gives no statements on the frequency of this behaviour. On the other hand, host egg abortion without successful parasitoid emergence is described as a mostly underestimated aspect of the regulatory capacity of egg parasitoids (ABRAM et al., 2016).

4.2.2 Egg parasitization by *Anastatus disparis*

Both the high consistency with which *A. disparis* emerged from egg masses and the high overall egg parasitization rate (19.2 %) **(Fig. 15)** were highly surprising and remarkable, as few comparable results are reported in Central Europe. FUESTER et al. (1983) observed *A. disparis* at all twelve study sites in Burgenland, but unfortunately did not provide any information on the abundance. HOCH (1995) did not find a single egg parasitoid in two years, despite a significantly larger sample than in the present study. The most recent Austrian study by KALBACHER (2008) did not investigate egg parasitism.

ALALOUNI et al. (2014) recently observed egg parasitization in two successive latency years in a German population. Although parasitization affected 42 % and 45 % of egg masses, only 1.2 % and 1.7% of the eggs were parasitized. CAMERINI (2009) made similar observations in Italy, where 19 % and 33 % of egg masses showed signs of parasitization by *A. disparis* in two successive years in a latent population. ZÚBRIK and NOVOTNÝ (1997) performed a large study on egg parasitization in Slovakia in the 1990s and collected more than 60,000 eggs. Nevertheless, the absolute number of emerging parasitoids was lower than from 4,433 eggs in the present study. The overall egg parasitization rate observed was 1.2 % and was caused by *A. disparis* in 95 % of cases. Parasitization rates tended to be highest during retrogradation. However, out of 22 site-year-combinations, only a single sample exceeded a rate of 2.0 %. The sample in question had a parasitization rate of 10.1 % and was from a low-density population. The results of ZÚBRIK and NOVOTNÝ (1997) agree with the results of earlier Slovak studies (ZÚBRIK et al., 2021).

In the 1970s, *A. disparis* caused parasitization rates of 1.7 % to 18.1 % in Serbia. Parasitization rates were lower in the same regions in the 1990s, and *O. kuvanae* clearly became the dominant species (MILANOVIČ et al., 1998). The high egg parasitization rates reported from Turkey were almost exclusively caused by *O. kuvanae*, while *A. disparis* was only observed sporadically (AVCI, 2009).

Higher parasitization rates by *A. disparis* in Europe were observed in the first half of the 20th century. KURIR (1944) found a maximum of 32.1 % parasitization in a Croatian population of high density. Furthermore, *A. disparis* emerged from 18.2 % of the eggs at another study site. In this case, also the unknown egg mortality rate of 26.1 % was in line with the results of the present study. However, such high parasitization rates were also exceptional in the study of KURIR (1944). For the remaining 16 site-year-combinations, the parasitization rates reached 0.4 % - 7.0 % or no wasps emerged in 61 % of the cases. BURGESS and CROSSMAN (1929) obtained *A. disparis* from 25 % of the eggs from Romania and Poland and reported average parasitization rates of 15 % in Spain.

Since the local and periodical oscillation in the abundance of *A. disparis* is well known (BURGESS and CROSSMAN, 1929; WELLENSTEIN and SCHWENKE, 1978), the significance of results from a single study site and year is limited. One factor that may have contributed to the high abundance in Eggenburg is the phase of the population cycle, since *A. disparis* typically is most abundant during retrogradation (KURIR, 1944; WELLENSTEIN and SCHWENKE, 1978; BATHON, 1993; ZÚBRIK and NOVOTNÝ, 1997). The female-bias in the sex ratio indicated favourable conditions for *A. disparis* and a very good temporal coincidence with the gypsy moth oviposition in summer 2019. In many cases, male-biased sex-ratios are observed, because the host eggs are already several days old at the time of parasitization (BATHON, 1993). A high host density probably facilitates the search for suitable host eggs. Since lower layers of eggs in the egg mass cannot be reached by the short ovipositor of the parasitic wasp (ZÚBRIK and NOVOTNÝ, 1997), a low number of layers in small egg masses enables higher parasitization rates.

Since high abundance of *A. disparis* was reported especially from more southern regions of Europe in the past, climatic changes might also be involved in the increased abundance in Austria. Between March and August, the mean monthly temperature in the study region in the period 2011-2020 was higher in each month than between 1981 and 2010. On average, the monthly mean temperature increased by 1.3 °C **(Fig. 48)** (ZAMG, 2020a). This might provide more favourable climatic conditions for *A. disparis* in Austria than in the past.

The calculation of the marginal infestation rate of *A. disparis* resulted in a value of 0.228, which corresponds to a theoretical value of 22.8 % reproductive egg mortality from *A. disparis*. The value exceeds the observed parasitization rate because parasitized eggs are exposed to the same environmental factors as non-parasitized eggs. Consequently, pre-mature death of *A. disparis* must also be expected to a distinct level. However, even the marginal infestation rate and *k*-value do not (or only to a limited extent) consider the non-reproductive impact of *A. disparis* on *L. dispar* eggs. Considering the data from KURIR (1944), ZÚBRIK and NOVOTNÝ (1997) and the present study, a positive linear correlation of medium strength (Pearson coefficient: r = 0.674) can be observed between the egg parasitization rate from *A. disparis* and the egg mortality due to unknown causes. In a regression model, 45.4 % of the variation of unknown egg mortality can be explained by the level of egg parasitization ($R^2 = 0.454$). This possibly indicates an additional, non-reproductive input from *A. disparis* on egg mortality, e.g., by host-feeding or by egg abortion. However, the correlation may also be caused by other factors, e.g., the increased abundance of *A. disparis* in retrogradation periods.

4.3 Larval and larval-pupal parasitoids

In the most recent studies from Central Europe, peak parasitism was not observed until the first year after the gypsy moth population densities returned to latency levels. When the host population density was still high and comparable to the level observed in this study, parasitization rates were significantly lower, particularly in younger instars. The parasitization rates ranged around 0.5-14 % in young larvae (L1 and L2), 2-14 % in middle-aged larvae (L3+L4) and 17-36 % in old larvae (L5 and L6) (HOCH et al., 2001; TURCÁNI et al., 2001; KALBACHER, 2008). Apparently, in the present study, parasitoids of young and middle-aged larvae contributed more to the collapse of the gypsy moth population.

4.3.1 Braconidae

Just as surprising as the high abundance of *G. porthetriae* in this study was the negligible role of *G. liparidis*. *Glyptapanteles porthetriae* has never been observed as the dominant braconid parasitoid of *L. dispar* in Austria. Preceding studies reported a dominance of *G. liparidis* (FUESTER et al., 1983; HOCH, 1995), *C. melanoscela* (EICHHORN, 1996) or a low total parasitization rate by braconids (KALBACHER, 2008). However, the majority of these investigations – except KALBACHER (2008) – were carried out in times of low gypsy moth population density and an increasing relative importance of *G. porthetriae* among braconids is well known (MAKSIMOVIĆ and SIVČEC, 1984; ČAPEK, 1988; ALALOUNI et al., 2013).

Glyptapanteles porthetriae

In addition to egg mortality, the most notable results of this study undoubtedly concern *G. porthetriae*. According to the calculated *k*-values, *G. porthetriae* had a higher generation effect on *L. dispar* than any other mortality factor **(Fig. 44)**. The calculated probability that an individual will survive the impact of *G. porthetriae* **(Equation 7)** was 39 %, which would correspond to a reduction of the larval population of approximately 61 %.

In most studies investigating European gypsy moth populations during retrogradation, *G. porthetriae* played a negligible role in the population collapse or was not present at all (FUESTER et al., 1983; MAIER, 1990; HOCH et al., 2001; TURCÁNI et al., 2001; ZOLUBAS et al., 2001; SUKOVATA and FUESTER, 2005; KALBACHER, 2008). A high abundance of *G. porthetriae* during retrogradation was reported from several populations in eastern and south-eastern Europe in the early 20th century (BURGESS and CROSSMAN, 1929), as well as in former Yugoslavia in the second half of the last century (MAKSIMOVIĆ and SIVČEC, 1984; FUESTER and RAMASESHIAH, 1989). In Austria, *G. porthetriae* has never been reported as the dominant parasitoid species. *Glyptapanteles porthetriae* was not found in the study of EICHHORN (1996) and was found at very low levels by FUESTER et al. (1983) and KALBACHER (2008). HOCH et al. (2001) observed the highest abundance during the latency periods, but even in this case the parasitization rates did not exceed 18 % (L1 and L2) or 9 % (L3 and L4) and were markedly lower at most sites.

The high parasitization rates in middle-aged larvae are particularly noteworthy, since *G. porthetriae* is described as a parasitoid mainly of the first two instars (GRIFFITHS, 1976; MARKTL et al., 2002) and it is assumed that only the spring generation of *G. porthetriae* attacks *L. dispar* (BURGESS and CROSSMAN, 1929; FUESTER et al., 1983; ČAPEK, 1988). This study provides evidence that the second/summer generation also parasitized gypsy moth larvae at the study site. This explains the significant increase in the parasitization rate in middle-aged larvae in the mid of June, after the eclosion of adult wasps obtained from young host larvae in late May and early June **(Fig. 50)**. The period of approximately two weeks

between the first peak of wasp eclosion and the second peak of the emergence of parasitoid larvae from the hosts corresponds to the endoparasitic development time of *G. porthetriae*, which is twelve to 14 days at 20 °C (NUSSBAUMER and SCHOPF, 2000). The long period over which *G. porthetriae* egressed from host larvae (calendar weeks 21-27) and the bimodal temporal distribution in the time of host death and eclosion of adult wasps **(Fig. 50)** support this assumption. In the studies by HOCH (1995) and KALBACHER (2008), *G. porthetriae* larvae almost exclusively emerged from host caterpillars in mid to late May, predominantly in calendar weeks 20 and 21. In 1994, one parasitoid individual emerged from the host in late June, which probably also originated from a second-generation parent.

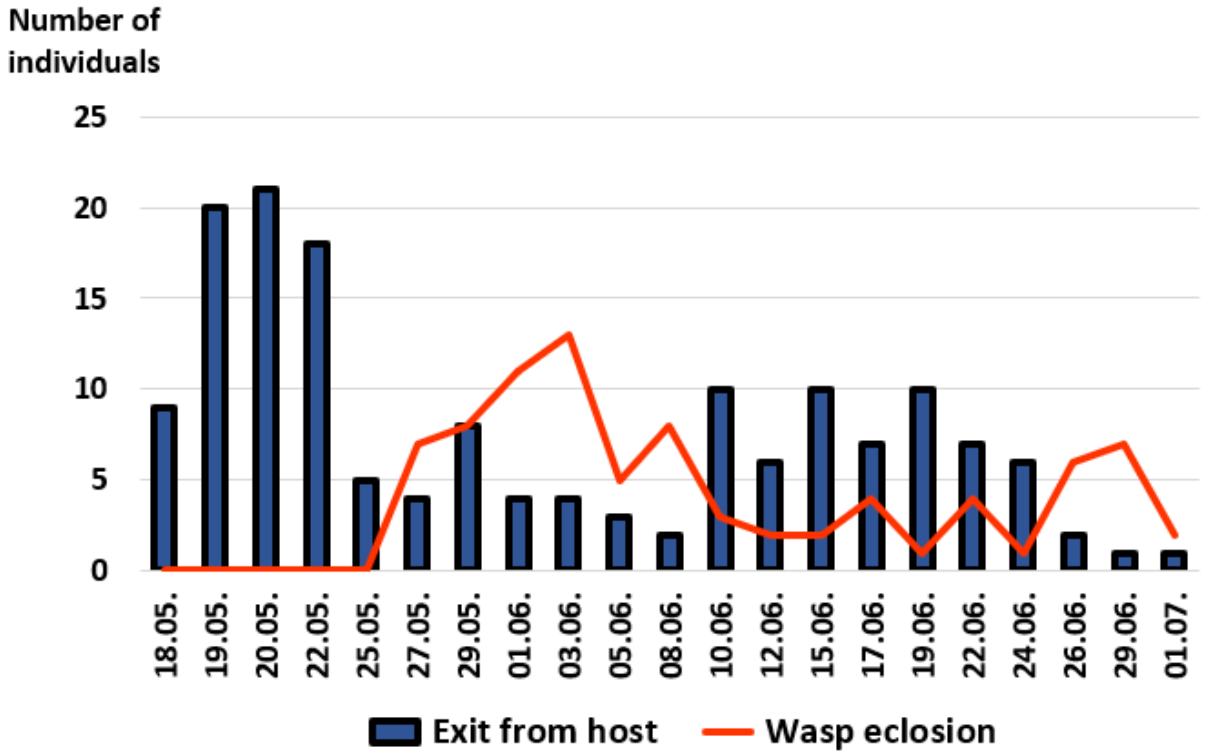

Fig. 50: Temporal sequence of *G. porthetriae* emergence from *L. dispar* host larvae and adult wasp eclosion from cocoons.

The most likely reason for the infestation of *L. dispar* by the second generation of *G. porthetriae* observed in the present study is the delayed phenology of young gypsy moth larvae. HOCH (1995) and KALBACHER (2008) do not provide any information on the time of *G. porthetriae* adult wasp eclosion, but the presence of second-generation wasps can be assumed from around two weeks after the egression from the host larva. This would correspond to calendar weeks 22-23. At that time, second instar host larvae were very rarely present and late third instars occasionally. In most cases, *L. dispar* had already reached the fourth or even later instars (**FIG. 47**). In the present study, the presence of adult wasps of the first/spring generation of *G. porthetriae* peaked in calendar weeks 22 and 23. In contrast to previous studies, L1 and L2 larvae were present until calendar week 22 and 23, respectively, and the presence of L3 larvae peaked at this time. Since the host suitability for *G. porthetriae* significantly decreases during the third instar of *L. dispar* (NUSSBAUMER and SCHOPF, 2000), the simultaneous presence of suitable gypsy moth host larvae and second-generation wasps of *G. porthetriae* seems to be an exception, caused by the low temperatures in May. JARZEMBOWSKA (2016) calculated a value of 6.9 °C as the lower development threshold (LDT) for the endoparasitic development of *G. porthetriae*, while the lower threshold for second instar gypsy moth larvae is around 8.5 °C (TROTTER et al., 2020). In the study region, the average daily minimum temperatures of 9.7 °C in May (as averaged values from Krems and Retz) usually exceed the

LDT for young gypsy moth larvae (ZAMG, 2021). In 2020, a mean daily minimum of 7.3 °C was measured at the study site, which is below the LDT for *L. dispar* but above the LDT for *G. porthetriae*. While in May only 9 % of the hourly measured temperatures were below the LDT for *G. porthetriae*, the temperature was below the LDT in 22 % of the time for gypsy moth larvae. Thus, temperature was probably at a level at which the development of *L. dispar* was disproportionally more impaired than the development of *G. porthetriae*.

In spite of the high parasitization rates in middle-aged larvae in the present study, the results partially agree with previous findings on host suitability (NUSSBAUMER and SCHOPF, 2000). Among the larvae collected on the same date, L3 larvae showed significantly higher parasitization rates than L4 larvae, which indicates the preference for the third instar **(Fig. 51)**. Furthermore, the significantly lower adult wasp emergence rate and the male-biased sex ratio of the wasps obtained from host larvae collected as mid-instars indicate their poor host suitability. Considering this, parasitization of *L. dispar* larvae by the second/summer wasp generation of *G. porthetriae* does not seem to be a promising strategy for the maintenance of the wasp population and was likely caused by a lack of more suitable hosts.

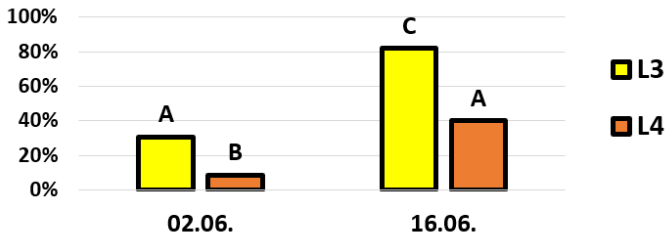

Fig. 51: Apparent parasitization rates by *G. porthetriae* of larvae collected on June 2nd and June 16th in third and fourth instar, respectively. Letters above columns represent statistical significances (Fisher's exact test, $p < 0.05$).

On the other hand, the parasitization success in the present study was surprisingly high. In the laboratory study by NUSSBAUMER and SCHOPF (2000), parasitoids emerged successfully in 68 % of L1 hosts and only 17 % of L3 hosts. The significant increase in apparent mortality in early June, when L2 larvae were only present in exceptional cases **(Fig. 11)**, indicates that most host larvae parasitized by the second generation of *G. porthetriae* were at least already in their third instar. Nevertheless, the parasitization success in these larvae in the field was apparently higher than in preferred host instars in the laboratory **(Fig. 51)**. A major factor contributing to a lower host suitability is the increased effectiveness of the cellular immune system of older *L. dispar* larvae against parasitoids (NUSSBAUMER and SCHOPF, 2000). Possibly, a reduced fitness of the gypsy moth population after two consecutive years of defoliation correlated with a reduced cellular immune response. In North America, HAJEK and VAN NOUHUYS (2016) observed high rates of possibly synergistic co-infestations of NPV and *C. melanoscela* in the field. They hypothesized that each species could benefit from the other species' lowering of the host's immune response.

Another factor that is possibly involved in the acceptance of middle-aged *L. dispar* larvae as hosts in the present study is that the availability of suitable alternative hosts likely did not meet the requirements of the high population density of *G. porthetriae*. Since the gypsy moth population had already significantly decreased in the early larval instars, it is possible that even gypsy moth larvae were rare as hosts. This might have contributed to a high rate of superparasitism and increased parasitization success. Other lepidopteran species as possible alternative hosts were not observed in conspicuous numbers at the study site.

Several individuals of the four-spotted footman, *Lithosia quadra* (L.) (Lep. Erebidae), were collected in the beating nets at the first collection date in the mid of May. An attempt was made to rear two individuals using the gypsy moth rearing protocol, but they did not develop, and

both died after approximately one month after collection. In the field, *L. quadra* was occasionally observed until mid of June. The rusty tussock moth, *Orgyia antiqua* (L.) (Lep., Erebidae) was occasionally found in June. The North American congener of this species – *Orgyia leucostigma* (Smith) – allowed successful development of *G. porthetriae* in laboratory experiments (RAFFA, 1977). *Lithosia quadra* hibernates as larvae (PATOČKA, 1980). Although *O. antiqua* usually overwinters in the egg stage, several authors have also reported overwintering as young larvae (JAHN and KOTSCHY, 1973). Both species, *L. quadra* and *O. antiqua*, may possibly be alternative overwintering hosts for *G. porthetriae*. However, there is no evidence or contradiction for this hypothesis in the literature.

Glyptapanteles liparidis

DORFMANN (2020) collected cocoons from *G. liparidis* at the study site in 2019. Although no quantitative studies on the parasitization rates were done, the abundance of *G. liparidis* was undoubtedly higher in 2019 than in 2020. However, he observed a very low hatching success and a high level of hyperparasitism. *G. liparidis* emerged from only 3 % of the field-collected cocoons, while hyperparasitoids – mainly chalcidids – emerged from 34 % of the cocoons and 64 % showed no eclosion at all. This could be a reason for the low abundance of the parasitoids in 2020. However, the field collections of the first generation of *G. porthetriae* cocoons in the present study showed similar rates of successful wasp eclosion and hyperparasitism. Nevertheless, the second generation of *G. porthetriae* was apparently still able to cause high parasitization rates in middle-aged host larvae.

Another possible explanation for the low abundance of *G. liparidis* is the lack of suitable alternative hibernation hosts. However, with the presence of *Pinus sylvestris* – the preferred host plant of the alternative host *Dendrolimus pini* – the study site provides at least one aspect, which was observed to be beneficial for overwintering of *G. liparidis* (FUESTER et al., 1983; ČAPEK, 1988).

The parasitization rates of the second *G. liparidis* generation often exceed those of the first generation (FUESTER et al., 1983), since the parasitoid population increases with a decrease in the host population. However, the unusual acceptance of *L. dispar* as a host for *G. porthetriae* wasps of the second generation probably also contributed to the low abundance of *G. liparidis*, which competes for host larvae with wasps of the summer generation of *G. porthetriae*. Wasps of the hibernating generation of *G. porthetriae* and *G. liparidis* probably eclose around the same time in spring. Although the difference in the endoparasitic development between *G. porthetriae* and *G. liparidis* is only 3 days at a constant temperature of 20 °C (MARKTL et al., 2002), the difference increases with decreasing temperature. With 9.3 °C, the LDT for the endoparasitic development of *G. liparidis* is markedly higher than for *G. porthetriae* (6.9 °C) (JARZEMBOWSKA, 2016), and also higher than for young gypsy moth larvae (8.5 °C) (TROTTER et al., 2020). The number of degree hours representing the effective temperature sum for endoparasitic development of *G. liparidis* in May reached only 59 % of the value for *G. porthetriae*. As a result, adult *G. liparidis* wasps of the summer generation probably eclosed much later than the second generation of *G. porthetriae*. Thus, many potential host larvae for the *G. liparidis* summer generation have either already been killed by *G. porthetriae* or *G. porthetriae* had at least already parasitized them. Even in the latter case, attacks by *G. liparids* would not be very promising, since the sequence of parasitization seems to be the major determinant of the competitive superiority between the two species (MARKTL et al., 2002).

Possibly, the fitness of the *G. liparidis* population at the study site has suffered from the defoliation in 2018 and 2019. While the hosts of *G. porthetriae* were probably not directly

affected by food shortage, larval development of *G. liparidis* often extends until the final host instars are present and defoliation progresses. In the present study, the only individual of the second generation killed its host in mid-July. HOCH (1995) observed emergence from the host by the second generation between late June and early July. The defoliation of the study site in the preceding years occurred already in June. The number of eggs oviposited by *G. liparidis* females into a host is adjusted depending on the size of the host (SCHOPF, 2007). At the time of oviposition, females are probably not able to predict the approaching food shortage for their hosts. This may result in an inadequate supply of resources for each of the gregarious parasitoid larvae, with possible consequences from reduced fertility of the emerging parasitoids up to host death without parasitoids emerging.

Cotesia melanoscela

C. melanoscela was detected inconsistently in previous Austrian studies. High parasitization rates were only observed with low populations densities (FUESTER et al., 1983; EICHHORN, 1996; HOCH et al., 2001). Considering the high population density and the very high abundance of hyperparasitoids of braconids at the study site, the low abundance of *C. melanoscela* is not surprising.

4.3.2 Ichneumonidae

Ichneumonids do not seem to play an important role as gypsy moth parasitoids in Central Europe, but members of two genera are often found. The present results are similar to the results of previous studies (FUESTER et al., 1983; EICHHORN, 1996; HOCH et al., 2001; TURCÁNI et al., 2001; KALBACHER, 2008). Although high parasitization rates from ichneumonids were reported in post-culmination periods, those were usually not reached before the population density had already reached a low level again. The most consistently occurring genus was *Phobocampe* in previous studies, which was not observed in the present study.

Hyposoter tricoloripes

Hyposoter tricoloripes (Viereck), *Hyposoter lymantriae* Cushman (GUPTA, 1983), *Hyposoter vierecki* Townes, Momoi and Townes (FUESTER and RAMASHESHIAH, 1989) and *Hyposoter fugitivus* Say (HOWARD and FISKE, 1911) are described as parasitoids of *L. dispar*. However, *H. lymantriae* is rather a parasitoid of the Indian gypsy moth, *Lymantria obfuscata* (GUPTA, 1983; FUESTER and RAMASHESHIAH, 1989; FUESTER and TAYLOR, 1991), a species closely related to *L. dispar*, but restricted to India and the Himalayas (DHARMADHIKARI et al., 1985). *Hyposoter vierecki* is a Far East congener of *H. tricoloripes* (FUESTER and RAMASHESHIAH, 1989). No report for parasitization of *L. dispar* by *H. fugitivus* was found, except of HOWARD and FISKE (1911), which refers to a single specimen, only determined on the base of a hyperparasitized cocoon. Older sources (e.g., BURGESS and CROSSMAN, 1929) also describe *Hyposoter disparis* Viereck, however this name refers to a species classified as *Phobocampe unicincta* (Gravenhorst) today, which must not be confused with the genus *Hyposoter* Förster (GUPTA, 1983). The recent review of *L. dispar* parasitoids by ŽIKIĆ et al. (2017) lists *H. tricoloripes* as the sole parasitoid of *L. dispar* within the genus *Hyposoter* in Europe. I follow this opinion and consequently did not distinguish between *H. tricoloripes* and *Hyposoter* spp. in this study, in contrast to e.g., HOCH et al. (2001).

Hyposoter tricoloripes prefers the youngest instars of *L. dispar* as a host (FUSCO, 1981) and the present results confirm that successful parasitization is possible from the first instar. However, larvae collected as L1 and L2 did not die until 3-4 weeks after collection, which was

significantly longer than the host death from *G. porthetriae*. This probably correlates with the competitive superiority of the braconid over *H. tricoloripes* if host parasitization by both species occurs in immediate succession. This would explain why the parasitization rates by *H. tricoloripes* were significantly higher in larvae collected in mid instars, despite the preference for young larvae. Host death of larvae collected as L3 and L4 took significantly less time and the host death occurred within a narrow time window, regardless of the stage of collection (L1-L4). This indicates that in the samples of middle-aged larvae parasitization occurred already in younger stages, too. Host death in the third or fourth instar supported previous field observations (FUESTER et al., 1981; FUESTER et al., 1983; HOCH, 1995). Old host larvae do not seem to be parasitized successfully.

4.3.3 Tachinidae

Tachinids undoubtedly contributed significantly to the collapse of the population towards the end of the generation cycle. However, such high parasitization rates by tachinids would not have been possible without the preceding significant reduction in the population density through other mortality agents – particularly braconids and pathogens. The present results on apparent mortality by *P. silvestris* support the current knowledge about the crucial role of this species in the collapse of *L. dispar* outbreak populations.

Parasetigena silvestris

The flight behaviour of *P. silvestris* is reported to depend strongly on weather conditions. The optimal temperature for flight ranges around 15-25 °C, (HERTING, 1960). The optimal temperature for oviposition is reported to range from 14-20 °C (PRELL, 1915) to 18-20 °C (VON FINCK, 1939) or above 20 °C (GOULD et al., 1992). In the present study, the temperature appeared to have little importance for the oviposition activity of *P. silvestris*, this becomes apparent when comparing the weather conditions prior to the collection dates on June 2nd and June 16th **(Fig. 52)**.

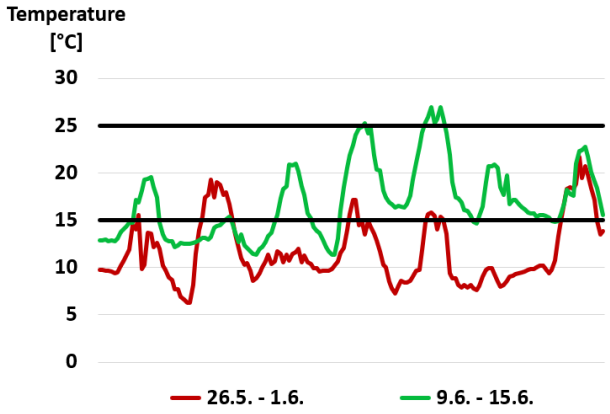

Fig. 52: Course of hourly temperature measured at the study site in the weeks before the collection dates, on June 2nd (red) and June 16th (green).
The black lines represent the optimal range for flight activity of *P. silvestris*, according to HERTING (1960).

In the week before June 2nd, the average daily mean temperature was 11.7°C and the temperature exceeded 15 °C for a total of 31 hours that week. In contrast, the daily temperature mean in the week before June 16th was 16.9 °C and provided 97 hours within the optimal range for flight activity of 15-25 °C. Von FINCK (1939) observed oviposition rates of *P. silvestris* females at temperatures of 16-18 °C that were twice as high as those at 10-12 °C. Nevertheless, the proportion of larvae that carried *P. silvestris* macrotype eggs did not differ significantly between the collection dates. On the contrary, L3 larvae collected on June 2nd had the highest number of larvae with *P. silvestris* eggs in the present study (38.7 %). The proportion of egg carrying L4 larvae collected on June 2nd was low (2.2 %); however, this was probably caused by the fact that most of the L4 larvae were freshly moulted at this point **(Fig. 11)**. NIKLAS (1939) stated that the temperature played a subordinate role for the flight and egg-laying activity of *P. silvestris*, while it was primarily

stimulated by the light intensity, and also the humidity played a major role. However, no significant differences in light intensity were measured in the weeks before June 2nd and 16th.

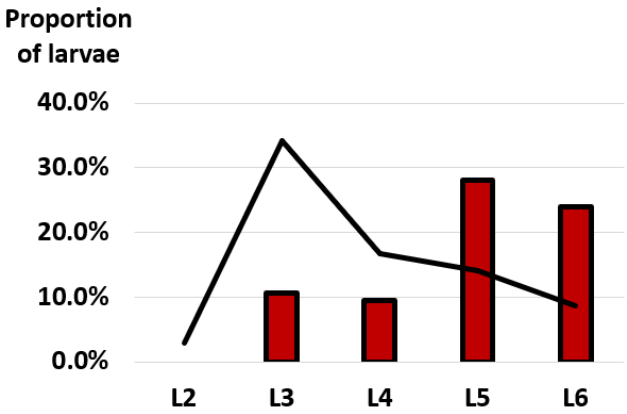

Fig. 53: Percentage of larvae with macrotype tachinid eggs at the time of collection (black line) and apparent mortality rates from *P. silvestris* depending on the collection-stage of *L. dispar* larvae.

Although larvae bearing macrotype tachinid eggs at the time of collection showed significantly higher parasitization rates by *P. silvestris*, the apparent parasitization success after oviposition varied significantly depending on the instar at the time of oviposition. Oviposition on middle-aged larvae obviously resulted in low parasitization success **(Fig. 53)**. The oviposition on second-instar larvae is probably underestimated, as they were only collected on a single collection date before the peak of flight activity. A second sample of L2 larvae in early June would probably have increased the stage-specific oviposition rate significantly. In previous studies too, a preference for younger larvae (L2, L3) and a decrease of oviposition from the fourth instar onwards were observed. This is mainly attributed to the change in the diel periodicity of *L. dispar* larvae from the fourth instar, when they begin to hide in their resting places during the period of the highest flight activity of *P. silvestris* (WESELOH, 1976; MAIER, 1990; LEE and PEMBERTON, 2019). There is also unanimous report that the first two gypsy moth instars are not suitable as hosts for *P. silvestris*. However, statements about the host suitability of L3 larvae are contradictory. GODWIN and ODELL (1984) report that premature host death before successful maggot emergence occurs consistently after invasion of L3 larvae and in most cases after invasion of L4 larvae in laboratory experiments. LEE and PEMBERTON (2019) did not observe successful parasitization of L3 larvae in the field. In contrast, MAIER (1990) reports 75.5 % mortality due to *P. silvestris* in L2 and L3 larvae bearing macrotype eggs at the time of collection. PEMBERTON et al. (1993) observed successful parasitization of larvae collected as L1 and L2 by *P. silvestris* in exceptional cases. HOCH et al. (2001) observed successful emergence from the third instar onwards.

In the present study there are indications that successful parasitization of host occurred from the second instar onwards and that the parasitization success in larvae oviposited in the third instar was higher than in the fourth instar. Only 12 % of the L4 larvae apparently killed by *P. silvestris* were bearing a tachinid egg at the time of collection. This indicates that most of them were already invaded by the parasitoid maggot in the third instar, since the empty egg chorion remains on the host cuticle until the next moult (PRELL, 1915). This is supported by a strong divergence between the oviposition rates and the apparent parasitization rates of the larvae collected in the fourth instar. While only 2 % of the L4 larvae collected on June 2nd were bearing tachinid eggs at the time of collection, this sample showed an apparent mortality rate from *P. silvestris* of 16 %. In contrast, 33 % of the L4 larvae in the samples from June 16th and 23rd carried tachinid eggs, while *P. silvestris* maggots emerged from only 3 % of the larvae in this sample. Hence, a highly significant lower oviposition rate resulted in a significantly higher parasitization rate. On the other hand, 25 % of the L3-collected larvae killed by *P. silvestris* did not carry a tachinid egg at the time of collection. These larvae have probably been invaded by the maggot already in an earlier stage.

The most likely explanation for these results can be found in the phenology of *L. dispar*. The time between moults generally increases significantly in later instars. This correlates with an increased probability that host moult occurs before the *P. silvestris* maggots hatch after oviposition (MAIER, 1990). The delay in the development of early instars in the present study increased the chance of successful invasion of *P. silvestris* maggots in younger hosts. The hatching of *P. silvestris* maggots is also delayed by low temperature, but PRELL (1915) reports a duration of three days at an average temperature of 20 °C, as opposed to six days at 12-14 °C. For *L. dispar*, the length of the first instar increases from 7-10 days at 20 °C to 14-17 days at 15 °C and 51-78 days at 10 °C (LIMBU et al., 2017), i.e., disproportionally stronger. An increase in temperature in June – at the same time as the appearance of L4 larvae in the field **(Fig. 11)** – accelerated the larval development and the fourth larval stage was passed very rapidly. This possibly led to a high proportion of tachinid eggs, which were stripped off prior to egg hatching **(Fig. 54)**. Gypsy moth larvae also passed the third instar quickly, but in contrast to the L4 larvae they were exposed to a cold period at the end of May. This was probably the major time of invasion of L3 larvae by *P. silvestris* maggots, as the L3 larvae collected on June 2nd not only showed the highest proportion of larvae with tachinid eggs, but also a significantly higher mortality from *P. silvestris* than the L3 larvae that were collected on the other dates. Oviposition on old larvae (L5 and L6) was rarely observed, but apparently resulted in high invasion rates and parasitization success. *P. silvestris* maggots emerged from more than 60 % of egg-bearing larvae of this group, in contrast to 20 % of middle-aged larvae (L3 and L4). On the other hand, the low proportion of egg-bearing larvae in samples of old instars indicates that also in this group oviposition and invasion might in many cases have already taken place in earlier stages.

Fig. 54: Freshly moulted L4 gypsy moth larva (right) and *Parasetigena* egg stripped off with the exuvia (left).

The significantly increased *G. porthetriae* parasitization rate in larvae bearing tachinid eggs provides strong evidence for the competitive superiority of the braconid over the tachinid species, as it was already observed by e.g., MAIER (1990). In addition to the poorer host suitability of middle-aged larvae, this was probably a major factor contributing to the lower mortality from *P. silvestris* in this group than in older larvae. Host larvae, which reached the fifth instar, were no longer exposed to the competition by braconids. The main competitor for old host larvae was *B. pratensis*. The competitive superiority between *P. silvestris* and *B. pratensis* is primarily determined by the sequence of attack (GODWIN and ODELL, 1981; MAIER, 1990). The results of the present study indicate a superiority of *P. silvestris*, since *B. pratensis* was hardly emerged form larvae with eggs of *P. silvestris* **(Tab. 5)**. Although the reduction in mortality due to *B. pratensis* in larvae bearing *P. silvestris* eggs was not significant at the stage-specific level, a clear trend was visible. The lack of significance was probably caused by the small group sizes of L3 and L4 larvae that were killed by *B. pratensis* and L5 and L6 larvae that were bearing *P. silvestris* eggs, which reduces the informative value of the statistical test. The competitive superiority of *P. silvestris* would in turn support the assumption that most invasions by *P. silvestris* already occurred in middle-aged larvae and not in the final instars when *B. pratensis* invasions most probably occurred. Otherwise, a more balanced ratio of competitive superiority could be expected.

Significantly increased total parasitism and mortality rates from a braconid species (*C. melanoscela*) in larvae bearing eggs of *P. silvestris* was also observed by LEE and PEMBERTON (2019). Parasitization by *G. porthetriae* correlates with a prolonged final instar before the host dies (NUSSBAUMER and SCHOPF, 2000), as it is the case with *C. melanoscela*. LEE and PEMBERTON (2019) assume the extended exposure to *P. silvestris* as the main factor for the correlation between observed tachinid eggs and mortality rates from braconids. I agree with that, but I think that there is also a sampling bias involved. A prolongation of the instar reduces the chance to strip the egg off by moulting and increases the chance that the larva will be sampled within this instar. Furthermore, many members of Microgastrinae induce behavioural changes in their hosts, which often move to more conspicuous and exposed places (QUICKE, 2015). This would also increase exposure to *P. silvestris* attacks.

Blepharipa pratensis

The constant mortality rate of approximately 5 % caused by *B. pratensis* in larvae collected in the first four instars is interesting, but in these cases, there is a high probability that parasitization did not occur in the field. Firstly, GODWIN and ODELL (1981) report that oviposition does not start until most host larvae have reached at least the third instar. Due to the delayed development of young larvae, this may have been different in the present study. But even if microtype eggs were present at the same time as young gypsy moth larvae, the chances of consuming eggs seem low. In the first two instars only 0.6 % (females) to 2.5 % (males) of the total amount of food are consumed by *L. dispar* larvae, while approximately 80 % are fed in the final instar (LEONARD, 1966). The probability for the uptake of *B. pratensis* eggs depends on the leaf area ingested. If *B. pratensis* eggs are ingested orally by L2 larvae, 90 % of the egg maggots are fatally crushed. Of the remaining 10 % of maggots, 98 % die prematurely in L2 hosts (GODWIN and ODELL, 1981). In the present study, 16 % of L1- and L2-collected larvae that pupated successfully, were subsequently killed by *B. pratensis*. Given the low chance that *B. pratensis* will develop successfully in young hosts, it seems highly improbable that this high proportion of killed pupae is a result of field invasions.

Instead, it seems more likely that *B. pratensis* mortality in larvae collected as early instars was due to egg-contaminated leaves used during larval rearing. Oak twigs used as food during the rearing were collected from the lower crown and care was taken to ensure that the leaves were not damaged by feeding. Although *B. pratensis* prefers leaves with feeding damage in the upper crown for oviposition (GODWIN and ODELL, 1981), leaves are chosen rather indiscriminately during the peak of oviposition (ODELL and GODWIN, 1984). The survival of microtype tachinid eggs mostly ranges around 7-30 (up to 75) days, depending on the tachinid species and humidity. Species that lay dark and hard-shelled eggs – such as *B. pratensis* – usually survive longer (HERTING, 1960). If *B. pratensis* eggs are ingested from the fourth instar of *L. dispar*, the probability for an undamaged uptake of the egg maggot increases to about 60 %, of which more than half usually survive their further development (GODWIN and ODELL, 1981). Thus, for larvae collected in young stages, this way of invasion seems much more likely than field invasions.

For larvae collected in mid-instars, the way of invasion is less clear. In the field, third and fourth instar larvae are often invaded at high rates. This was shown by MAIER (1990), who dissected middle-aged and old larvae and checked the presence of maggots. Although the invasion rates were significantly higher in L5 and L6 larvae, *B. pratensis* maggots were also found in 40 % of L3 and L4 larvae. Nevertheless, I assume that in the present study third and fourth instar larvae were rather invaded during rearing. Both the apparent mortality from *B. pratensis* and the mortality of successfully pupated larvae were very similar from the first to the fourth instar.

The significant increase in mortality from the fifth to the sixth instar supports previous knowledge about the increased effectiveness against female gypsy moth larvae (SABROSKY and REARDON, 1976; FUESTER and TAYLOR, 1996). This can easily be explained by the higher food uptake of females. The food consumption of sixth-instar females corresponds to more than three times the food uptake during the entire development of male larvae. Overall, the food uptake of females is approximately four times higher (LEONARD, 1966). On the other hand, most of the L5-collected individuals killed by *B. pratensis* were likely to be males, as 91 % of them pupated after the fifth instar. The sex of the pupae was not determined, but 87 % of the surviving *L. dispar* individuals that pupated after five instars were male. A disproportional impact on males in this group can be explained by food uptake, but primarily by sample bias. Food uptake is about 50 % higher in the last (L5) male instar than in the penultimate female instar (L5). In addition, males remain in the fifth instar about twice as long as females with an additional instar (LEONARD, 1966), which increased the chance of being sampled.

The competitiveness of *Blepharipa* sp. seems to be weak due to the very late onset of its actual development. This was shown impressively by MAIER (1990). While dissections of *L. dispar* larvae showed that 80 % were invaded by *Blepharipa* sp. maggots (mainly *B. schineri*), mature maggots emerged out from only 3 % of the individuals of the same samples. On the other hand, 43 % were killed *by P. silvestris*. In samples collected in July, about 90 % showed multiparasitism and were infested with maggots of both tachinid species. In the present study, invasion by *B. pratensis* probably reached similar values at the end of the season, at least in female larvae. This is indicated by a 70 % mortality rate among the L6-collected individuals that pupated successfully. For L5-collected larvae, this value reached 37 % and may provide an indication of the invasion rates in the males. In contrast, the abundance of *P. silvestris* in the present study seems to be markedly lower than in the study by MAIER (1990). This is indicated by a significantly lower rate of larvae bearing *P. silvestris* eggs in the present study. Interestingly, the lower abundance of *P. silvestris* apparently had a higher impact on the mortality from *Blepharipa* sp. than from *P. silvestris* as the apparent mortality by the latter is much more similar between the studies. I suspect that the lower abundance of the competitively superior *P. silvestris* in the present study enabled a higher mortality from *B. pratensis*.

Despite the low level of competitiveness, *Blepharipa* sp. seems to play an important and very special role among the natural enemies of *L. dispar*, particularly in the final collapse of outbreak populations. In my opinion, during retrogradation *Blepharipa* sp. resembles a kind of boss character in a video game, lurking in the background and providing one last large hurdle for gypsy moth larvae that have survived the impact of other mortality factors. The high reproductive capacity and longevity of eggs allow *B. pratensis* to appear almost anywhere where other mortality agents did not kill the host. During the gradation periods, defoliation probably impairs the oviposition strategy. Therefore, its abundance is usually lower in these periods (ALALOUNI et al., 2013), although its reproductive capacity is the highest of the major parasitoids in Central Europe.

Samples of L6 larvae must be considered with caution, as they are almost exclusively female individuals. Hence, they cannot be compared directly to samples from other instars, because they are based on different statistical populations. Therefore, mortality from *B. pratensis* is probably better represented by the marginal infestation rate and *k*-value than by stage-specific mortality rates and peak parasitism. The peak mortality from *B. pratensis* clearly exceeds the peak mortality from *P. silvestris*, but only a small proportion of the individuals reached the sixth instar in the male-biased population. In contrast, the marginal infestation rate is higher for *P. silvestris*, which affects both sexes to a similar extent. According to **Equation 7**, the probability

that a *L. dispar* individual survives the impact of *B. pratensis* is 51 %, for *P. silvestris* it is 44 %. I assume, both values are good estimates for their regulative capacity.
Although the regulatory influence of *B. pratensis* on the present generation may be smaller, the disproportionate impact of *B. pratensis* on females probably correlates with a stronger impact on the subsequent generation of *L. dispar* in the following year.

4.4 Hyperparasitism of primary gypsy moth parasitoids

High levels of hyperparasitism by a broad spectrum of hyperparasitic wasps were observed for the braconid *G. porthetriae*. Obviously, eggs or larvae of *G. porthetriae* were already parasitized to a low extent when they were still developing inside their gypsy moth host. This was shown by the eclosion of hyperparasitoids from braconid cocoons obtained from field-collected larvae **(Fig. 33)**. Members of the ichneumonid subfamily Mesochorinae, a group of obligatory hyperparasitoids with a mostly wide host range, are known to typically inject their eggs into their host larvae, while these are concealed in the haemocoel of the primary host (WAHL, 1993; SCHWENKE, 1999).

A much higher mortality rate of *G. porthetriae* was caused by so-called pseudohyperparasitoids, which attack cocoons of primary parasitoids **(Fig. 34)** (QUICKE, 2015). In many cases, it was probably one of the same species that had been observed at the study site the year before as hyperparasitoids of *G. liparidis* cocoons (DORFMANN, 2020) and probably contributed to the low abundance of *G. liparidis* observed in the present study. However, this raises the question why *G. liparidis* was putatively more affected by hyperparasitoids than *G. porthetriae* in 2019. A possible explanation can be found in the gregarious lifestyle and the emergence from older host larvae. *G. liparidis* often kills hosts as late instars (SCHOPF and HOCH, 1997), *G. porthetriae* usually as second or third instars. This possibly increases the chance that *G. liparidis* larvae will emerge from hosts during their resting time, e.g., on the tree trunk. The emergence of *G. porthetriae* from its host larva is probably more common in the higher parts of the tree crown, where young larvae are typically found. Among other hyperparasitoids, particularly pteromalids and some wingless *Gelis* species – both were observed in the present study – prefer the lower parts of the tree for the host search (GODFRAY, 1994). This may have resulted in a higher exposure of *G. liparidis* cocoons and thus an increased risk of reproductive as well as non-reproductive mortality from them, since many chalcidid hyperparasitoids also show extensive host-feed behaviour (GODFRAY, 1994). Furthermore, cocoon clusters of the gregarious species may be more attractive to hyperparasitoids than solitary cocoons with scattered distribution. Sampling of *G. porthetriae* cocoons from the ground can also lead to an overestimation of hyperparasitism.

Puparia of *B. pratensis* also suffered high mortality from hyperparasitoids. Since only puparia from field-collected gypsy moth larvae or pupae were examined, hyperparasitoid attacks evidently occurred within the host in all cases and the mortality rates do not take into account possible attacks during the long time of exposure of the hibernating puparia to natural enemies in the soil. Females of the perilampid *P. ruficornis* lay their eggs on leaves, where they are ingested by gypsy moth caterpillars. The hyperparasitoid larvae then penetrate any tachinid maggots that may be present but show no further development until the maggot leaves the host caterpillar and pupates. After pupation of the tachinid maggot, the *P. ruficornis* larvae egress the pupa and feed externally on their host within its puparium **(Fig. 32)**. The development to the adult wasp already occurs in the same summer in which the gypsy moth caterpillar was killed, and the adult wasps hibernate within the puparium of their tachinid host (MAIER, 1990).

4.5 Impact of pathogens and unknown mortality factors

4.5.1 Mortality of larvae collected as first instars

The apparent causes of mortality differed markedly between larvae collected as L1 compared to larvae collected in any other instar. While first-instar larvae were hardly attacked by parasitoids, the apparent mortality from pathogens and unknown causes was high in this group. However, death from these causes was often delayed and occurred in later stages, which is reflected in the low stage-specific *k*-value for L1 larvae **(Fig. 43)**.

According to YERGER and ROSSITER (1996), neonate larvae usually die within less than 14 days after NPV infections and within less than seven days in the case of developmental disorders caused by other factors than pathogens. However, their study was conducted at a constant temperature of 23 °C, which allows for faster development than at field temperature. In the present study, only 16 % of the larvae killed by unknown factors died within the first week after collection. This indicates that in many cases undetected pathogens were probably also involved in the death of these larvae.
The dominant role of NPV among confirmed pathogen deaths suggests that NPV may also be dominant in these cases. This assumption is supported by the temporal sequence of host death from undetermined causes in larvae collected in the first instar **(Fig. 55)**, which is very similar to the bimodal distribution observed in larvae killed by NPV. A high proportion of the undetected pathogen mortality in young larvae can be explained by their small body size, which makes the collection of tissue samples difficult. Furthermore, a lower concentration of pathogen units may be sufficient to kill the vulnerable early instars.

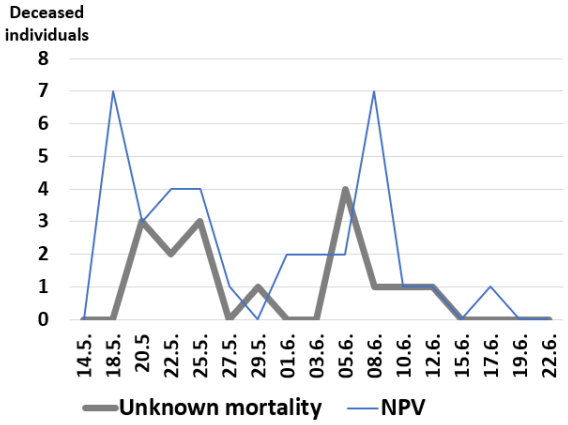

Fig. 55: Temporal sequence of mortality of L1 larvae collected on May 14th due to NPV and unspecified causes.

On the other hand, only 51 % of the L1-collected larvae, which were evidently killed by pathogens, died within 14 days of collection. Additionally, it must be considered that the date of collection in this study was very likely not the day of infection and field infections would have occurred earlier. The late host death, as well as the bimodal temporal distribution of host death **(Fig. 55)** indicate that a considerable proportion of the pathogen infections did not occur in the field, but rather during the group-rearing of young larvae. This is supported by the fact that 63 % of NPV mortalities after 14 days or later occurred in two out of ten (i.e., 20 %) of the rearing boxes. These two rearing boxes showed a highly significant accumulation of late NPV deaths compared to the other rearing boxes. The bimodal temporal distribution of NPV mortality with two very clear peaks suggests a division into NPV deaths induced by field infections and deaths induced by infections during group rearing. Almost 50 % of the L1-collected larvae died in June, constituting the second peak, and were likely infected during rearing. The remaining 53 % killed in May are probably due to field infections. Accordingly, the apparent mortality from NPV in larvae collected in the first instar would decrease by 16.1 percentage points to 18.2 % if only these cases are considered, which probably result from field infections. This value still exceeds the apparent mortality rates for all other collection stages significantly with the exception of L5.

This adjusted mortality rate agrees very well with the stage-specific marginal infestation rate of NPV, which was according to **Eq. 4** calculated as 19.2 % in the first instar and thus was significantly lower than the apparent mortality rate **(Fig. 56A)**. A similar picture could be observed for the larval mortality due to unknown causes **(Fig. 56B)**. The apparent mortality rate exceeded the marginal infestation rate significantly in L1 larvae and slightly in L2 larvae also in this case.

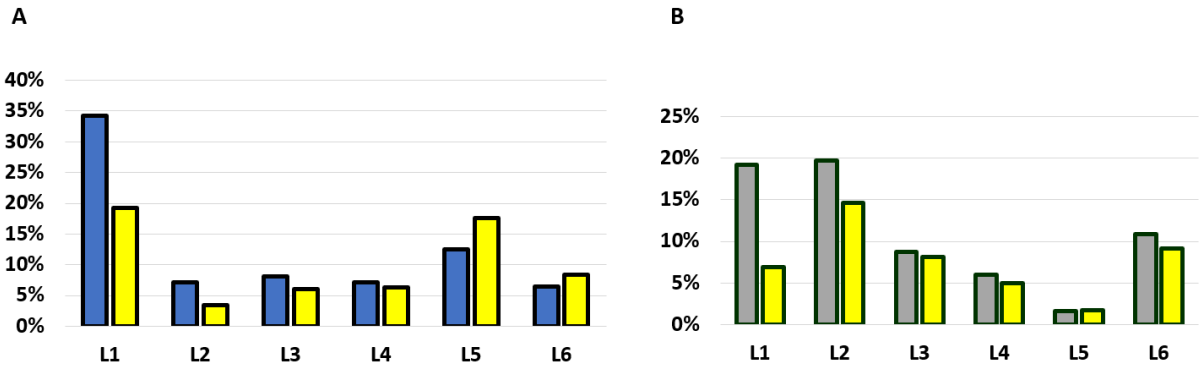

Fig. 56: Comparison of stage-specific apparent *L. dispar* mortality rates (blue/grey) and marginal attack rates (yellow) for:
A) NPV.
B) Unknown larval mortality.

Infections during rearing can explain the strong divergence between the apparent mortality rate from NPV and the corresponding infestation attack rate. Due to the delayed death and the preceding development progress prior to death, infections during rearing had little influence on the marginal infestation rates, which was probably a very robust measure against this bias. If the unknown mortality in the first instar is mainly due to undetected NPV infections, this would also explain the divergence between apparent mortality and marginal infestation rates for unknown mortality factors in this group **(Fig. 56B)**. This of course, would in turn correlate with an underestimation of the NPV mortality. In my opinion, the sum of the marginal infestation rates for NPV (19.2 %) and unknown mortality (6.9 %) can be used for the realistic assumption that field mortality from NPV in the first instar did not significantly exceed 25 %, at least at the time of collection of the L1 and L2 larvae. Of course, cadavers can be an inoculum for new infections in the field, however, these are included in follow-up samplings. Furthermore, *L. dispar* larvae are able to recognize and avoid NPV-infected cadavers (PARKER et al., 2010). In the small rearing boxes, this possibility was probably strongly impaired.
L2-collected larvae were also reared in groups until their first moult. However, with an average time of 10 days to death or transfer to single rearing, this time was significantly shorter than the 16 days for L1 larvae (t-test: $p < 0.001$). Although second instars probably also experienced some infections during rearing, the ratio appeared to be significantly lower. L2 larvae showed neither a bimodal distribution of NPV or unknown mortality, nor such a strong divergence between apparent mortality rates and marginal infestation rates.

4.5.2 NPV

According to the calculated *k*-values, NPV was the fourth most effective mortality factor in the present study, with a killing power comparable to that of *B. pratensis* **(Fig. 44).** The probability that a *L. dispar* individual survived the impact of NPV was calculated to be 52 % **(Eq. 7)**.
HOCH et al. (2019) investigated the pathogen mortality at the study site in June 2018 and June 2019. In 2018 – the first year of the population outbreak – the NPV prevalence was very low with 1.8 % apparent mortality in old larvae. In June 2019, an apparent mortality of 58.4 % was observed. However, this sample was collected after *E. maimaiga* had already caused high mortality in the gypsy moth population. As a result, NPV mortality was undoubtedly overestimated when compared to the samples in the present study, which were collected on multiple dates. Nevertheless, NPV in 2019 was probably more prevalent at the study site than in 2020. The higher NPV mortality of old larvae in 2019 probably correlated with defoliation. Starvation was shown to increase the susceptibility of larvae to NPV and can probably trigger latent infections (PAVLUSHIN et al., 2019).

The possibility of infections during rearing and the more sophisticated evidence of pathogen mortality result in a lower comparability of different studies as for mortality from parasitoids. In contrast to previous studies investigating the collapse of outbreak populations, NPV mortality appeared to be relatively high in young larvae, but low in old larvae (MAIER, 1990; HOCH et al., 2001; TURCÁNI et al., 2001; HOCH et al., 2019). Due to longer exposure time (REARDON and PODGWAITE, 1976; GODWIN and SHIELDS, 1984) and the increasing contamination of the environment with occlusion bodies, the NPV prevalence typically increases with larval age (MURRAY et al., 1989) and the second wave of the bimodal infection cycle typically causes higher mortality rates (WOODS and ELKINTON, 1987).

No significant increase in the NPV mortality of middle-aged to old larvae was observed in the present study. The weather conditions in 2020 may have contributed to that; however, their role in the epizootiology of NPV is difficult to assess (D'AMICO and ELKINTON, 1995). High temperatures at the time of egg hatching – which coincided with the warm April 2020 – correlate positively with NPV prevalence (HAJEK and TOBIN, 2011). However, the main abiotic factor impacting the epizootic dynamics of NPVs is probably sunlight, which can inactivate occlusion bodies completely within 24 hours (MENT et al., 2018). April 2020 was the second sunniest April ever measured in Austria. With a surplus of 63 % in sunshine duration compared to the long-term average, the surplus in Lower Austria was particularly high (ZAMG, 2020c). NPV-infected larvae tend to die at elevated positions in the crown (MURRAY and ELKINTON, 1992), leading to increased exposure to the sun, particularly if the canopy is not fully developed in spring. This can be mitigated by rainfalls, which move occlusion bodies downwards to more shady areas of the crown (MENT et al., 2018), but April 2020 was also very dry (ZAMG, 2020c). These weather conditions may have resulted in a high rate of inactivation of occlusion bodies derived from cadavers of neonate larvae infected in the first cycle of infection, and hence a reduced inoculum for further infections.

In addition to strong differences in NPV susceptibility within an instar (McNEIL et al., 2010) and between different instars, susceptibility is primarily dependent on body weight. The lethal dose for fifth-instar larvae is more than 100-fold higher than for first-instar larvae (SHAPIRO et al., 1986). There is no clear evidence for sex-specific differences in NPV susceptibility and both male-biased (AKHANAEV et al., 2020) as well as female-biased (IL'INYKH et al., 2009) sex ratios were observed in survivors from populations treated with intermediate doses of NPV. In the present study, the NPV mortality in L5 larvae was almost twice that of L6 larvae, although the difference was not significant. This may be caused in part by a higher number of undetected infections in L6 larvae, which would explain the higher unknown mortality rates within this

group. On the other hand, infections of older female larvae possibly did not result in larval mortality in many cases but rather in undetected sublethal NPV infections.

The generation effect of direct larval mortality from NPV – represented by the *k*-value **(Fig. 44)** – is exceeded by three parasitoid species. However, simply considering direct mortality is likely to greatly underestimate the impact of NPV (as well as other pathogens). In females that survived NPV infections fecundity is reduced by 21-74 % and the hatching rate of larvae is reduced by 11-63 %. Furthermore, effects on fecundity and natality are not restricted to directly infected females but have also been observed in their progenies (MYERS et al., 2000; IL'INYKH et al., 2009; AKHANAEV et al., 2020). Considering these indirect effects, NPV probably had a higher impact on the collapse of the gypsy moth population than any parasitoid species.

4.5.3 *Entomophaga maimaiga*

In June 2019, *E. maimaiga* was found to be the cause of mortality in 65 % of 165 larval cadavers collected in the field at the study site but caused only 2 % apparent mortality in 89 individuals collected as living larvae at the same time and reared until pupation. In contrast, NPV was detected as the cause of death in 4 % of the cadavers but killed 58 % of the living larvae during rearing (HOCH et al., 2019). The high divergence between cadavers and living larvae appears unusual but can be explained by several reasons. The intention of the cited study was to detect *E. maimaiga* by sampling cadavers on the stem basis and not to draw representative samples to measure the prevalence of pathogens. Furthermore, HAJEK and TOBIN (2011) found that *E. maimaiga* is up to 200 times more likely to be detected in field-collected cadavers than in field-collected larvae, and the divergence can be explained with the premortal behaviour of larvae infected with *E. maimaiga* or NPV. While cadavers of late-instar larvae killed by *E. maimaiga* are usually attached to the bark of the tree trunk (HAJEK, 1999), NPV-infected larvae show a climbing behaviour immediately before death and mostly die in the upper canopy (MURRAY and ELKINTON, 1992). As a result, *E. maimaiga* is overrepresented in cadavers collected using conventional techniques, because cadavers are much easier to find. On the other hand, the time window for collecting infected living larvae is significantly longer for NPV than for *E. maimaiga* (MALAKAR et al., 1999), which leads to an overrepresentation of NPV, particularly if samples are taken on a single date, as was the case by HOCH et al. (2019). Considering this, the prevalence of *E. maimaiga* and NPV at the study site in 2019 was probably at a similar and high level for both pathogens and neither of them clearly dominated.

In 2020, cadavers of larvae were observed only in exceptional cases and not sampled. Samples were taken weekly from the end of June. This corresponds roughly to the minimum duration of an infection cycle by *E. maimaiga* (MALAKAR et al., 1999) and hence, the underrepresentation of *E. maimaiga* in samples of living larvae was probably largely overcome and the samples provide a reliable measure of the prevalence of *E. maimaiga*. From the fourth instar, no significant differences in stage-specific apparent mortality rates between NPV and *E. maimaiga* were observed. This indicates an almost balanced prevalence of both pathogens in the old larvae in 2020 as well, although at a significantly lower level than in 2019.

The reason for the low prevalence of *E. maimaiga* is probably the dry weather in spring 2020 **(Fig. 48)**. Soil moisture and relative air humidity correlate positively with initial infections by azygospores and with the formation, discharge, and germination of conidia. Under field conditions, resting spores usually begin to germinate one to two weeks before the larvae hatch and continue to germinate until mid to late June. However, this requires sufficient humidity (HAJEK, 1999). HAJEK and TOBIN (2011) found positive correlations for April precipitation

and first infections by azygospores, June temperature and secondary conidial infections as well as a strongly negative correlation between May temperature and first infections. April 2020 was very dry, which probably did not allow an initial infection of neonate larvae. After a humid week in the end of May **(Fig. 11)**, the first infection by *E. maimaiga* was detected in a larva collected on June 2nd. Subsequently the heavy rainfall on June 7th resulted in the significant increase of the mortality from *E. maimaiga* in larvae collected in the second half of June. Although L3 larvae were still present in the field during this humid period **(Fig. 11)**, detected infections by *E. maimaiga* were restricted to the fourth or older instars of the gypsy moth. This may correlate with the stage-specific behaviour of larvae. Neonate L1 larvae are frequently blown to the soil by wind and L4 to L6 larvae often rest in the ground litter during the day. Late L1, L2 and L3 instars have in common that they are rarely found on the ground (WESELOH, 1990), where probably most infections by azygospores occur (HAJEK, 1999; HAJEK, 2001). This probably reflects in the mortality rates of June 2nd and 16th, when on the one hand both L3 and older instars were collected and on the other hand, infections by *E. maimaiga* were detected. While *E. maimaiga* was not detected in any of the 99 L3 larvae collected on these dates, it was detected in 4 of 87 (4.6 %) L4 and L5 larvae. This difference is significant ($p = 0.046$) and indicates that no infections occurred before the fourth instar.

Apparently, the simultaneous occurrence of sufficient soil moisture and the presence of larval instars on the soil was necessary to initiate a primary infection by azygospores, and these requirements were not met until the last week of May or early June. This is supported by the exclusive detection of conidia in the L4 cadavers killed in mid-June, an indication of infections by germ conidia from azygospores (HAJEK, 1999). Such a late start of initial infections markedly reduces the number of possible infection cycles. Secondary infections from cadaver-borne conidia probably started no earlier than in mid-June but were likely the only source of infections from then on. This was indicated by the presence of conidia and azygospores in all larvae that died after June 23rd and agrees to the literature, according to which the germination activity of azygospores ceases between mid and late June (HAJEK, 1999). Although precipitation in June 2020 was high, this month probably did not offer optimal conditions for conidia germination, as the average temperature with 16.8 °C at the study site was relatively low. Subsequently, low rates of infection were observed until mid-July, but apparently the density of airborne conidia did not reach sufficiently high levels to cause high mortality rates in the already sparse population.

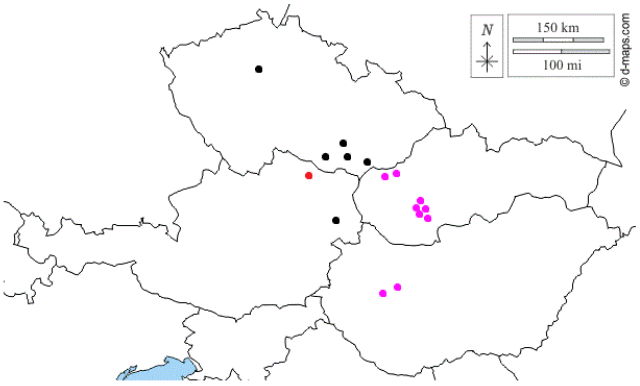

Fig. 57: Spread of *Entomophaga maimaiga*: Pink dots represent the most western localities with reported establishment of *E. maimaiga* before 2017 in Slovakia (ZÚBRIK et al., 2018) and Hungary (CSÓKA et al., 2014).
Black dots represent sites where E. maimaiga was first detected in 2019 (HOCH et al., 2019; HOLUŠA et al., 2020).
The red dot indicates the study site in Eggenburg.

In 2019, the first year *E. maimaiga* was detected in Austria, the weather conditions in April were very similar to April 2020 **(Fig. 48)**, while May apparently brought very favourable conditions for the fungus. May 2019 was one of the ten rainiest May-months ever measured in Austria since 1858 and the coolest May for more than 25 years (ZAMG, 2019). A precipitation excess of 100 % and an average temperature 2.7 °C below the long-term mean was observed in the study region (mean values for Krems and Retz, according to ZAMG (2020a)). In contrast to HAJEK and TOBIN (2011), who viewed April

precipitation as crucial for initial infections, HAJEK et al. (1996) observed positive correlations with precipitation in May.

Over medium distances, however, the spread of *E. maimaiga* into new areas of distribution occurs most probably by wind dispersion of airborne conidia and less often via azygospores, which are mainly spread by human activities but usually not by wind (HAJEK, 1999; ZÚBRIK et al., 2016; HOLUŠA et al., 2020). Hence, weather conditions in the neighbouring countries with *E. maimaiga* establishment and putatively wind dynamics can be assumed to be decisive. Extensive epizootics of *E. maimaiga* – predisposed by a cold and wet May – were reported from Slovakia in 2019, with gypsy moth mortality peaking in early June. The closest location with reported establishment of *E. maimaiga* before 2017 (Žliabky, SK: 48°22'N, 17°28'E) (ZÚBRIK et al., 2021) is approximately 120 km eastern of Eggenburg and in close vicinity to most sites where *E. maimaiga* was detected for the first time in the Czech Republic in 2019 (HOLUŠA et al., 2020) **(Fig. 57)**. A spread of *E. maimaiga* over distances of more than 100 km per year is reported from North America (HAJEK, 1999).
It seems very likely that conidia from cadavers in the epizootic in Slovakia provided the inoculum for the infections in Austria and the Czech Republic in the same year. The spread to Austria is not surprising and has already been expected by e.g., ZÚBRIK et al. (2018).

According to the *k*-value, *E. maimaiga* was the second most effective pathogen and the sixth most effective identified mortality factor in 2020 **(Fig. 44)**. The probability that a *L. dispar* individual survived the generation impact of *E. maimaiga* was calculated to be 83 % **(Eq. 7)**.

4.5.4 *Endoreticulatus schubergi*

Apparently only a single *L. dispar* larva was killed by *E. schubergi*, thus, the direct generation impact of the microsporidium on larval mortality was negligibly. Due to its low virulence, however, the prevalence of *E. schubergi* is probably greatly underestimated simply by considering apparent mortality. In laboratory experiments, 60 % of the *L. dispar* larvae emerged as adults after inoculation with *E. schubergi* in the third instar (GOERTZ and HOCH, 2008a). Infections are limited to epithelial midgut cells, which are continuously excreted by infected hosts (WEISER, 1998). This might impair the detection of light infections in contrast to pathogens that are present and accumulated throughout the haemocoel. Furthermore, spores are typically enveloped in spherical groups of mostly 16 or 32 spores **(Fig. 58B)** (MADDOX et al., 1996). These structures were disintegrated in the field-collected cadaver **(Fig. 58A)**, probably due to freezing. In the cadaver in question, the infection was nevertheless recognized, and the structures were observed in the larvae used for the bioassay to confirm the detection.

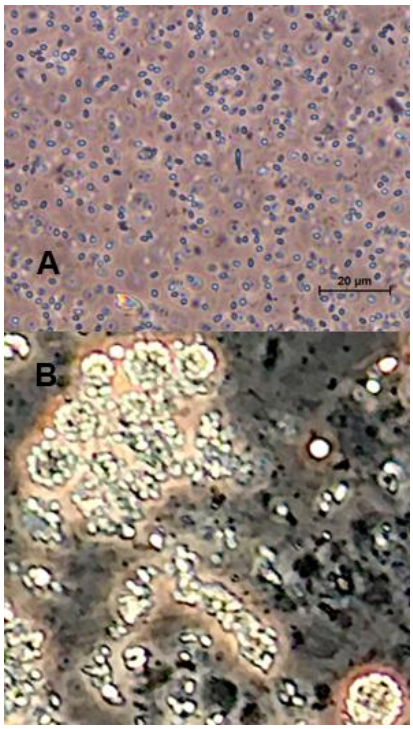

Fig. 58: Microscopic symptoms of larvae infected with *E. schubergi.*
A) Spores in the cadaver of the field-collected larva: Not clustered in characteristic structures.
B) Spores in the cadaver of larvae used for the bioassay: Spores enveloped in spherical groups.

An impairment of the host's nutritional metabolism by *E. schubergi* may cause host death during pupation (HOCH et al., 2009). *Endoreticulatus schubergi* may have been involved in the unknown pupal mortality, which affected 25 % of field-collected pupae and 10 % of pupae

from field-collected larvae. In any case, the sublethal effects of *E. schubergi* probably had more of an impact on the *L. dispar* population than direct mortality and could likely be one of the factors contributing to the low fecundity and natality of the gypsy moth population investigated.

Endoreticulatus schubergi is frequently observed at low levels in European gypsy moth populations (NOVOTNÝ et al., 1996; McMANUS and SOLTER, 2003; PILARSKA et al., 2006a). HOCH et al. (2001) consistently found microsporidia in Burgenland, mainly from middle-aged and old larvae. The apparent mortality rates ranged from 0-6.4 %.

4.5.5 Unknown fungi

Considerable amounts of fungal spores other than those of *E. maimaiga* were found in cadavers of larvae collected in all instars, except L5. Although no significant differences in the mortality from unknown fungi were found in individual comparisons of collection stages, young larvae (L1 and L2; 4.7%) showed a significantly higher ($p = 0.022$) mortality than older larvae (L3 to L6; 1.7%). All four L3 larvae killed by unknown fungi carried macrotype tachinid eggs and appeared to be heavily infested with *P. silvestris* as each larva carried an average of 2.25 eggs, compared to 1.19 eggs of the remaining L3 larvae with eggs, and 0.44 eggs in the total L3 sample. The difference is not significant when comparing larvae with eggs only ($p = 0.056$), but the difference becomes significant when comparing with the total L3 sample (0.016).

Numerous species of fungi have been isolated from *L. dispar* (WELLENSTEIN and SCHWENKE, 1978). *Beauveria bassiana* (Ascomycota, Hypocreales) is one of the most important fungal pathogens of *L. dispar* and is frequently isolated from gypsy moth larvae in Europe (WELLENSTEIN and SCHWENKE, 1978; NOVOTNÝ et al., 1996; CONTARINI et al., 2013; DRAGANOVA et al., 2013), Asia (SAEIDI, 2011; ALALOUNI et al., 2013) and North America (HAJEK et al., 1997; BLACKBURN and HAJEK, 2017). *Beauveria bassiana* has a very broad host range, literature data range from more than 200 (BLACKBURN and HAJEK, 2018) to more than 700 (SHAPIRO-ILAN et al., 2012) lepidopteran and coleopteran hosts. *Beauveria bassiana* typically causes mortality rates of less than 10 % in *L. dispar* populations (CONTARINI et al., 2013; DRAGANOVA et al., 2013; BLACKBURN and HAJEK, 2017). CONTARINI et al. (2013) report a prevalence of 17.5 % at a single study site in Italy.

With the exception of *E. maimaiga*, fungal spores in larval cadavers were not reliably identified and no isolation and cultivation was attempted in this study. However, the spores in some (but not all) cadavers were very similar to the spores of *B. bassiana* **(Fig. 59A-B)**, according to the descriptions by BLACKBURN and HAJEK (2018) and KOCH et al. (2018). The cadaver of the L6 larva began to sporulate very intensely and was covered by a thick layer of whitish spore powder the next day **(Fig. 59C)**. This corresponds to the macroscopic symptoms of old *L. dispar* larvae killed by B*. bassiana* (SAEIDI, 2011; DARA et al., 2019). It seems likely that *B. bassiana* was present at the study site and was responsible for most of the unknown fungal mortality.

Beauveria bassiana generally shows a low virulence to *L. dispar*, however, L1 and L2 larvae are more susceptible than larvae of the third instar (DRAGANOVA et al., 2013). This agrees with the higher mortality in young larvae observed in the present study. The significantly increased mortality from unknown fungi in L3 to L6 larvae bearing tachinid eggs compared to those without eggs indicates that predisposing factors for the fungus are necessary to kill older larvae. Parasitization is known to increase the susceptibility of hosts to entomopathogens (BROOKS, 1993).

The generation impact of direct mortality from unknown fungi was somewhat less than the impact of *E. maimaiga* **(Fig. 44)**. The probability that a *L. dispar* individual survived the generation impact of unidentified fungi was calculated to be 85 % **(Eq. 7)**.

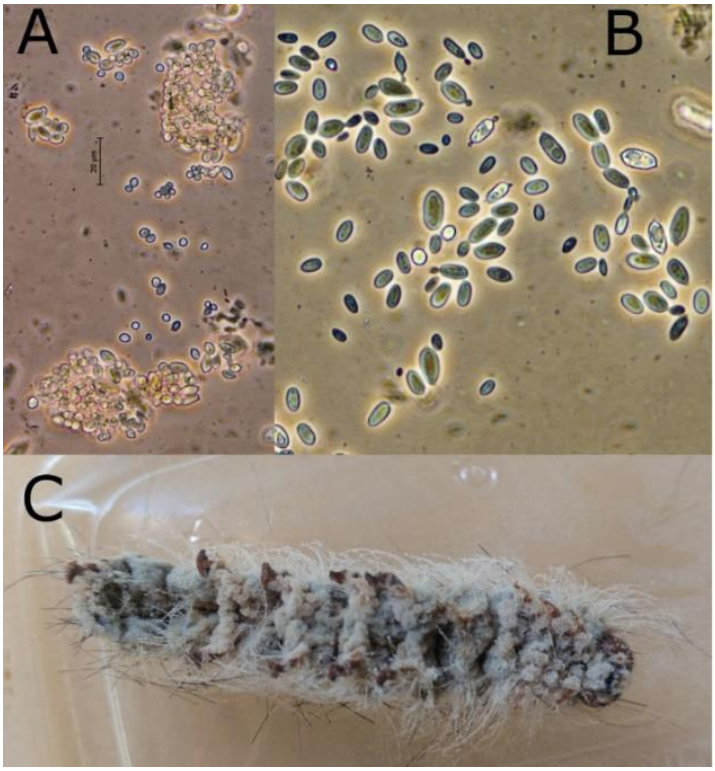

Fig. 59: Microscopic and macroscopic symptoms of larvae killed by unknown fungi, putatively *B. bassiana.*
A) Densely clustered globose spores, putatively conidia (2.8-4.5 x 2.0-3.2 µm).
B) Oblong, irregularly shaped spores, putatively blastospores (4.8-8.4 x 2.4-4.4 µm).
C) Intensive sporulation of the cadaver of an L6 larva, one day after death.

4.5.6 Unknown Mortality

Several factors may have contributed to the unknown mortality, including premature host death after parasitization or pseudoparasitization, undetected pathogen infections, physiological disorders, unfavourable rearing conditions, and in the case of eggs predatory activity. The mortality rates from undetected causes were similar to the rates in other comparable studies (e.g., HOCH et al., 2001; KALBACHER, 2008).

The unknown mortality was highest in young larvae (L1 and L2). A possible role of NPV has already been discussed **(Chapter 4.5.1)**. Furthermore, young larvae are more prone to starvation, unfavourable temperature, and humidity conditions, suffer higher mortality due to genotypic deficiencies (REARDON and PODGWAITE, 1976), and are more prone to bacterial diseases (NOVOTNÝ, 1989).

The generation impact of unknown mortality factors was comparable to the impact of the most important parasitoid species, *G. porthetriae* **(Fig. 44)**. However, the high *k*-value is mainly due to the high unknown egg mortality, which corresponds to 40 % of the total killing power **(Fig. 43)**. If only larval and pupal development is considered, the *k*-value of unknown mortality factors is ranked in the fourth place and is also exceeded by *P. silvestris*, *B. pratensis* and NPV.

4.6 Impact of predators

Predators are important natural enemies of all stages of the gypsy moth. Generalist predators are particularly important during low population densities (latency period). Birds are assumed to be the most important egg predators, causing mortality rates of around 30 % (ALALOUNI et al., 2013; ZÚBRIK et al., 2021). Small mammals – particularly mice – have been shown to cause high pupal mortality in Austrian *L. dispar* populations during the latency period, especially in forests with dense shrub vegetation (GSCHWANTNER et al., 2002). The most important invertebrate predator of larvae and pupae in Central Europe is the carabid beetle *Calosoma sycophanta* (L.), which is regularly observed in high abundance during gypsy moth outbreaks. Other invertebrate predators observed frequently but in lower abundance during outbreaks are *Calosoma inquisitor* (L.) (Col., Carabidae) and *Xylodrepa quadripunctata* (L.) (Col., Silphidae) (FUESTER et al., 1983; HOCH, 1995; WERMELINGER, 1995; KALBACHER, 2008). *Calosoma sycophanta* was highly abundant at the study site, while *C. inquisitor* and *X. quadripunctata* were not observed.

The population dynamics of *C. sycophanta* are closely related to the population dynamics of the gypsy moth (SPIELES an HORN, 1998). Adult beetles have a lifespan of three to four years and a low tendency for dispersion (WESELOH, 1985), while larval development is completed within approximately one month, usually at the time of gypsy moth pupation. Both adult beetles and larvae feed extensively on larvae and pupae of *L. dispar* (FUESTER et al., 2014). The reproduction of *C. sycophanta* is closely linked to prey density and if prey is scarce, adult beetles enter their overwintering sites in the soil after a few weeks in early summer without reproducing (SPIELES and HORN, 1998). *Calosoma sycophanta* is typically most abundant in the second or third year after the gypsy moth culmination (WESELOH, 1985).

While pupal mortality caused by *Calosoma* sp. is negligible during latency periods (GSCHWANTNER et al., 2002), it frequently causes high mortality rates during the culmination and retrogradation periods, particularly in pupae on the basal tree trunk. HOCH (1995) and KALBACHER (2008) quantified the predation rates by *Calosoma* sp. in Austrian gypsy moth outbreak populations and observed rates of 38-81 % in pupae on the trunk, while 13-15 % of pupae on lower branches were destroyed. Similar results are reported from North America (WESELOH, 1985).

The high abundance of *C. sycophanta* and the low abundance of gypsy moth pupae in the present study suggest high predation rates. Interestingly, no remains of damaged pupae could be observed, as is usually the case after predation of *Calosoma* sp. Rather it gave the impression that the pupae had disappeared. All of the few field-collected pupae were probably collected immediately after pupation. Neither the eclosion of adult moths nor the emergence of parasitoid was observed earlier than eleven days after collection and occurred on average on the fifteenth and eleventh day after collection, respectively. This corresponded exactly to the duration observed in pupae of field-collected larvae. Possibly, *C. sycophanta* has been outcompeted by other predators, e.g., mice, which sometimes remove pupae and store them (GSCHWANTNER et al., 2002) or the remains have fallen to the ground.

Specimens of *C. sycophanta* observed at the study site were all adults, while no larvae were observed. This indicates that reproduction has not or has rarely occurred, probably due to prey scarcity. This agrees with the observations made by FUESTER et al. (1983) in a collapsing Austrian population in the second year after culmination.

Ants (Hym., Formicidae) were ubiquitously at the study site and were occasionally observed to prey on young gypsy moth larvae. Ants are among the most important predators of young

larvae (WESELOH, 1990) and eggs (BROWN and CAMERON, 1982) in North America. Many European ant species avoid *L. dispar* larvae, while others – e.g., *Formica fusca*, a species that typically coexists with *L. dispar* – have been shown to prey on them (GOERTZ and HOCH, 2013). Few studies have been done on ants as predators of *L. dispar* in Europe. However, it has been shown that ants can be effective predators of the nun moth, *Lymantria monacha*. The results were inconsistent at all study sites, but in one case 95 % of the nun moth pupae placed on tree trunks were removed and collected from ants (ADLUNG, 1966). It is conceivable that ants have also removed the gypsy moth pupae at the study site.

4.7 *L. dispar* survivors

Interestingly, 20 % of the male survivors pupated after the fourth instar. No surviving female pupated after the L4 stage, however, 27 % of the females pupated after the fifth instar. A possible explanation is the incorrect determination of the instar. The determination was based on the width of the head capsule, which usually allows good discrimination between the fourth and fifth instar. In average, the head capsule is nearly 40 % wider in the fifth instar (WELLENSTEIN and SCHWENKE, 1978), but the difference may be smaller in malnourished larvae. Nevertheless, also other possible explanations should be considered.

Different populations of *L. dispar* commonly differ in their number of instars. However, the differences usually manifest as extra instars of males, females, or both, in addition to the common base of five male and five to six female instars. In contrast, a reduced number of instars appears rather unusual and was not observed in studies on North American gypsy moth populations (LEONARD, 1966). Populations with a proportion of males pupating after the fourth instar have been observed in Japan, although this was only in two out of 86 populations investigated. In one of them, males with four instars were reported only in one out of four study years (NAGASAWA, 1988).

At first glance it seems appropriate to attribute the reduced number of instars to unfavourable rearing conditions. However, it is unanimously reported that environmental conditions such as crowding, starvation during early instars, photoperiodic depression and unfavourable temperature and humidity lead to additional larval instars, but not to a reduced number of larval stages (LEONARD, 1970; LEONARD, 1974; LEONARD, 1981; NAGASAWA, 1988). In the present study, additional larval instars were rare, not a single larva developed with seven instars and only 2 % of the surviving males went through six instars.

Braconids (NUSSBAUMER et al., 2002; SCHAFELLNER et al., 2007) and microsporidia (KARLHOFER et al., 2012) also inhibit host pupation of *L. dispar* by impairment of the inactivation of juvenile hormone, while NPV induces the same effect by inactivation of ecdysteroids (BURAND and PARK, 1992). Consequently, an impact of the natural enemies present also cannot explain a reduced number of instars and precocious pupation.

Pupation after the fourth instar was not associated with increased pupal mortality, but it was slightly delayed and occurred on average eight days after the pupation of the males with five instars. The same effect was observed in females that pupated after five and six instars, respectively. Hence, genetic selection for faster larval development to avoid late instar mortality can also be excluded as a possible explanation. Eventually, the individuals with premature pupation overcame attacks by natural enemies, which delayed their development, and pupated after regaining hormonal balance.

4.8 Development of *Lymantria dispar* population density in 2020

Fig. 60 estimates the development of the field population density at the study site in 2020 and illustrates the high divergence in the host reservoir offered to the natural enemies by the various development stages of *L. dispar*. Unknown egg mortality and egg parasitization by *A. disparis* as well as NPV mortality in the first instar larvae and parasitization from *G. porthetriae* induced a sharp decrease in the absolute number of *L. dispar* individuals early in the season. In contrast, parasitoids of old larvae – particularly tachinids – caused higher mortality rates, which, however, affected a much lower absolute number of host individuals. Nevertheless, the impact of natural enemies in older stages should not be underestimated. Applying a sex ratio of 1:1 and the observed fecundity of 222 eggs per female, the estimated number of *L. dispar* individuals in the pupal stage would still result in an increased number of eggs in the following year. In contrast, the estimated number of adult moths results in a decrease in egg density when applied to the same calculation. In the latter case, the calculation estimates that the egg density in 2021 will reach a level of 57 % of the density in spring 2020. The complete collapse of the population observed in spring 2021 showed that the reduction of the population in the field was even stronger than calculated. However, this can be explained by the impact of predators, which was not included in the calculation. Thus, **Fig. 60** seems to give a realistic approximation for the influence of parasitoids and pathogens on the population dynamics of *L. dispar* in the field.

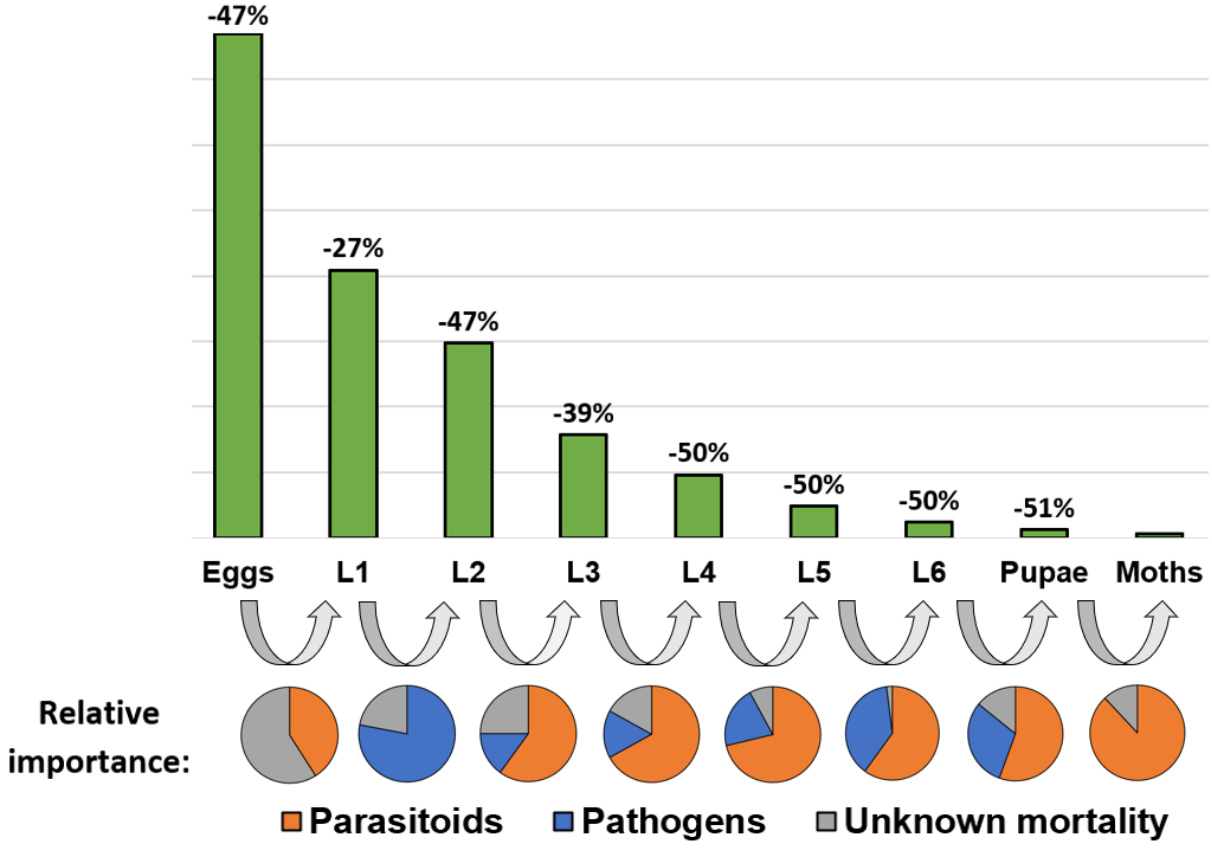

Fig. 60: Estimated progress in the reduction of the *L. dispar* field population at the study site in 2020.
Numbers above columns represent the proportion of individuals killed in the collection stage, calculated according to **Equation 8**. Pupal mortality is based on larvae collected in the last instar and pupae collected in the field (see **Equations 1 and 2**).

4.9 Discussion of methods

4.9.1 Impact of sample sizes and collection techniques

The intended sample size was 100 larvae per collection stage. While this was clearly overfulfilled for L2 and L3 larvae, the target for older larvae from the fourth instar was not achieved. However, sample sizes were within acceptable ranges for all stages except the field-collected pupae. While small sample sizes may possibly impair the resolution of the study to detect mortality factors occurring in low abundance, the larval sample sizes were likely large enough to reliably depict the impact of major mortality factors. Small sample sizes in older larvae were also partially offset by splitting of the collections over several collection dates. Strong divergences between larvae of the same instar, which were collected at different times, showed a strong influence of the collection date. Restricting the sampling of young larvae (L1 and L2) to a single date was probably a bigger problem than small sample sizes in old larvae. In particular, the sample of L1 larvae, which had a relatively small sample size, was collected on a single date, and subsequently probably suffered pathogen infections during group rearing must be viewed critically. For future studies, at least two collection dates per stage are recommended.

From late June burlap bands were used to collect the larvae. This technique can cause sampling bias. However, due to the low density of older larvae, its use was inevitable. REARDON (1976) observed significantly increased parasitism by *P. silvestris*, *B. pratensis* and *C. melanoscela* in larvae collected under burlap bands. MAIER (1990) observed that larvae under burlap bands were preferred by *P. silvestris* for oviposition. In both cases, however, the population density was relatively high, resulting in the accumulation of numerous larvae under each burlap band. On the other hand, GOULD et al. (1992) observed lower parasitization rates by *P. silvestris* under burlap bands, but the accumulation of eggs on some larvae, probably those that rested at the periphery of burlap bands. FUESTER et al. (1983) observed neither changes in parasitization rates nor in species composition depending on the collection technique. Due to the very sparse population of old larvae **(Fig. 46)** in the present study, the provision of burlap bands was not associated with clusters of larvae, which probably attract parasitoids. If the bands harboured larvae at all, the number only exceeded two larvae in exceptional cases. Therefore, I assume that the use of burlap bands in the present study did not have a significant impact on the parasitization rates.

Collection techniques may also have influenced the observed prevalence of pathogens. Late-instar larvae resting in the litter are exposed to a significantly higher risk of infections by *E. maimaiga* than larvae resting on the tree trunk (HAJEK, 2001). The provision of artificial resting sites on tree trunks may have reduced the proportion of larvae resting in the litter. The latter can also be underrepresented in samples collected from burlap bands. NPV prevalence was reported to be underestimated with burlap band collections. However, this is due to the premortal climbing behaviour, which is probably initiated only shortly before death (MURRAY and ELKINTON, 1992). Considering the slow disease progress of NPV in older larvae and the high frequency of collection dates, a high influence on the prevalence in late-instar larvae seems unlikely in the present study. However, this may be different for young larvae.

4.9.2 Statistical approaches

Both statistical approaches showed strengths and weaknesses in the present study and partially complemented each other. The method for calculating the marginal infestation rates and *k*-values requires only a few additional data compared to using the conventional apparent mortality rates. The results of both approaches largely agreed. If this was not the case, an

explanation was often found for it. Hence, a large discrepancy could be used as an indicator for e.g., potential methodological bias.

Marginal attack rates and *k*-values allowed a very clear and compact presentation and rough comparability of the influence of different mortality factors over the generation development of *L. dispar*. The method was shown to be very robust against several putative methodological artefacts, including pathogen infections during rearing (NPV), contaminated food (*B. pratensis*) and possible inclusion in multiple samples in the case of host developmental progress between attack and death. On the other hand, especially in the latter case also the calculation of marginal infestation rates and *k*-values had some limitations. In these cases, competition effects were poorly taken into account by the method and were sometimes totally ignored. For example, *H. tricoloripes* frequently attacked young larvae, but in many cases was probably outcompeted by braconids. Since no host death by *H. tricoloripes* occurred before the third host instar, this resulted in marginal infestation rates of zero for L1 and L2 larvae. The total killing power of *H. tricoloripes* was attributed to middle-aged larvae. In these cases, host death occurred fast in most cases. This led to the – supposedly wrong – assumption of high competitiveness and the assignment of a high *c*-value in the calculation. This was also similar with *B. pratensis*, the parasitoid that probably suffered most from competition from other mortality factors. Hence, the aim of separating the effects of simultaneously acting mortality factors largely failed. The stage of infestation – particularly the initial stage of infestation, i.e., the youngest stage that was attacked by a certain mortality agent – was better represented by the apparent mortality rates, with the exception of *B. pratensis*. Other effects, including sublethal effects from pathogens or non-reproductive mortality from parasitoids, were not accounted for by either method.

5 Outlook on future developments

Lymantria dispar is assumed to have benefited from climate change in many regions of Eurasia. Both the range of distribution and the outbreak foci have shifted northward in the recent decades, and these developments are expected to continue (VAHANEN et al., 2007; YASYUKEVICH et al., 2015). This may be linked to more frequent and extensive outbreaks in Austria in the future. However, due to the low proportion of oak trees in Austrian forests (2 %) and their fragmented distribution, Austria probably faces a lower risk than neighbouring countries, such as Slovakia (12 % oaks) or Hungary (33 %) (McMANUS and CSÓKA, 2007). Furthermore, *E. maimaiga* is expected to mitigate the intensity of future outbreaks in Central Europe (ZÚBRIK et al., 2018; ZÚBRIK et al., 2021). These assumptions are based on observations in south-eastern Europe, where several predicted outbreaks of *L. dispar* have not occurred since the fungus was established (PILARSKA et al., 2016).

With *A. disparis* and *G. porthetriae*, the most notable results of the present study concerned two parasitoid species, which in the past were most frequently observed in more southern parts of Europe. Future investigations on Austrian gypsy moth populations will show whether this is part of a sustainable development due to climatic changes or was only observed by chance in a study on a single population in a single year.

6 Summary

The gypsy moth (*Lymantria dispar* L.) (Lep., Erebidae) is an important pest in European oak forests. Populations of *L. dispar* typically follow periodic gradation cycles and the extensive complex of natural enemies of the gypsy moth is an important factor in the regulation of the population dynamics. In 2018, a mass outbreak of *L. dispar* was observed in Eggenburg (Lower Austria), which resulted in the defoliation of an oak forest in early summer 2018 and 2019. In 2020, the population density of the gypsy moth was still at a high level but was expected to decline. This offered the opportunity to investigate the role of parasitoids and pathogens of *L. dispar* in the decline of the mass outbreak.

From April to July 2020, in total 20 egg masses, 680 larvae, and twelve pupae of *L. dispar* were collected in the field and reared under semi-field conditions until the emergence of adult moths or until death. Larvae were fed with fresh oak leaves and checked for death three times weekly. Parasitization was detected by the presence of cocoons or puparia outside the host larvae. Cadavers of non-parasitized larvae were examined for the presence of pathogens with phase contrast microscopy. Mortality rates were calculated stage-specifically for eggs, all six instars (L1 to L6), and pupae of *L. dispar*.

Gypsy moth larvae emerged from 53 % of the collected eggs, while 28 % of the eggs showed no hatching and 19 % were parasitized by *Anastatus disparis* (Hym., Eupelmidae). The egg parasitization rate significantly exceeded the values reported in previous studies in Austria or neighbouring Central European countries.

Field-collected larvae were mainly killed by parasitoids. *Glyptapanteles porthetriae* (Hym., Braconidae) was the dominant parasitoid of young and middle-aged larvae (L1 to L4 instars). The highest mortality from this species was observed in larvae collected in the second instar (36 %), but *G. porthetriae* also emerged from more than 20 % of the larvae collected in the third and fourth instar. In contrast to previous studies in Austria, the mortality from *G. porthetriae* was markedly higher in the present study and there are indications that also the second generation of adult wasps attacked middle-aged *L. dispar* larvae in summer.

Parasetigena silvestris (Dip., Tachinidae) and *Blepharipa pratensis* (Dip., Tachinidae) were rarely observed in young and middle-aged larvae but were the dominant parasitoids of old larvae collected in the final instars (L5 and L6) and together caused parasitization rates of 48 % in L5 larvae and 61 % in L6 larvae, respectively. Both species were already observed frequently and highly abundant in previous studies in Austria.
Three further parasitoid species were observed at low abundance. *Hyposoter tricoloripes* (Hym., Ichneumonidae) caused stage-specific parasitization rates from 1 % (L1) to 8 % (L4). *Glyptapanteles liparidis* (Hym., Braconidae) emerged from two host larvae, *Cotesia* sp. (Hym., Braconidae) from one host larva.

While pathogens caused 41 % mortality in larvae collected in the first instar, the mortality rates decreased to 11-18 % in larvae collected from the second to the sixth instar. The Nuclear Polyhedrosis Virus (NPV) was the dominant pathogen in all instars, causing mortality rates of 7-34 %. The fungus *Entomophaga maimaiga*, which was recorded for the first time in Austria in 2019, played a minor role and the stage-specific mortality rates did not exceed 5 %. This was probably due to the dry spring in 2020, which inhibited the germination of *E. maimaiga* resting spores in the soil. The microsporidium *Endoreticulatus schubergi* was detected in one gypsy moth cadaver. Spores of unidentified fungi were detected in 3 % of all larvae collected and 13 % of all larvae died due to unknown causes.

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