flachen Schalen) sind bekannt. Literatur zu diesen Fragen und Methoden vergleiche[2].

Uns interessierte, ob die Prager Methode der Submerskultur von *B. bassiana*[3,4] sich besonders einfach mit einer Oberflächenkultur verbinden lasse, indem die Nährlösung mit den in ihr nach 3 Tagen gebildeten Blastosporen (etwa 6×10^8/ml) zur reichlichen Beimpfung von Weizenkleie benutzt wird. Die Submerskultur könnte so schnell und laufend reichlich Impfmaterial liefern, die Weizenkleie das Auskeimen der Blastosporen und Konidienbildung ermöglichen. Weizenkleie schien uns wegen ihrer Saugfähigkeit für das hoch dosierte Inoculum besonders geeignet, zumal sie als Kultursubstrat für den Pilz bekannt ist[1] und bei der Aufbereitung des Materials (wie Zerkleinern, Absieben) nicht stören dürfte.

Die Kleie wurde unsteril benutzt, da bei der hohen Einsaatmenge des Pilzes ein unterdrückender Effekt auf die natürliche Mikroflora der Kleie zu erwarten war. Das Resultat bestätigte unsere Annahme: Fremdpilze erschienen nicht. Soll das fertige Produkt aus hygienischen Gründen keinerlei Fremdkeime enthalten, lässt sich natürlich auch zuvor sterilisierte Kleie verwenden.

Ein Beispiel möge die Brauchbarkeit der Methode belegen. Unsterile Kleie wurde mit 3 Tage alter, Blastosporen-durchsetzter Submerskultur im Verhältnis 1:1 (Gew./Vol.) gemischt. Die erhaltene Paste wurde in Petrischalen ausgestrichen, die bei 20 °C im Labor oder bei 28 °C im Brutschrank aufgestellt wurden. Nach 48 Stunden wurden die Deckel der Schalen gelüftet. 14 Tage nach Ansetzen der Kulturen hatte sich auf der bei 20 °C gehaltenen Kleie reichlich Mycel entwickelt, die Sporulation war aber gering. Sie zeigte sich aber sehr stark auf der bei 28 °C gehaltenen Kleie. 1 cm³ Material (Kleie plus fruktifizierter Pilz) aus 20 °C enthielt $8{,}3 \times 10^9$, aus 28 °C dagegen $4{,}8 \times 10^{10}$ Konidien.

Man wird die Methode den jeweils vorhandenen Möglichkeiten und dem Pilzstamm anpassen, indem z. B. Lagerung der beimpften Kleie – ausgestrichen oder, bei Zusatz einer geringeren Menge Impfflüssigkeit, locker geschüttet –, Temperatur, Belüftung und Kulturdauer variiert werden. Uns kam es hier nur darauf an, auf ein für Massenkulturen mögliches Prinzip hinzuweisen, das sich ausser für *B. bassiana* auch für andere insektenpathogene Pilze eignen dürfte.

Summary. After incubation for 3 days, a submerged culture containing blastospores of *Beauveria bassiana* was mixed with unsterile wheat bran at the ratio of 1:1 (v/w). The paste was then spread to form a thin layer and stored for 2 weeks at 28 °C. This combination of culture methods resulted in a rich production of the relatively resistant conidia (4.8×10^{10}/cm³ paste medium) which are very suitable in experiments for biological control of insect pests.

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Two Cell-Free Systems for the Assay of Virus-Specific Polymerase

In the early stage of replication of RNA viruses virus specific polymerase (RNA-dependent RNA nucleotidyl transferase) is synthesized. This enzyme is coded by viral genome and it catalyzes the synthesis of viral RNA. The synthesis occurs in the presence of four nucleosidetriphosphates (ATP, GTP, UTP, CTP), ATP-regenerating system, Mg^{++} and SH-contining compounds.

In cells infected with Arboviruses[1,2] and Picornaviruses[3,4] virus specific polymerase is associated with the mitochondrial-microsomal (MM) fraction of the cells, that is sedimented at 12,000–15,000 *g*. The reaction reaches a plateau within 30–45 min and the main product synthesized is a double-stranded RNA.

We attempted to prolong the time-course of polymerase reaction and compared two polymerase systems for this purpose.

Experiments were conducted with the SPF strain of Venezuelan equine encephalomyelitis (VEE) virus propagated in chick embryo fibroblast monolayers[5]. The cells ($1–2 \times 10^6$ cells/ml) were infected with the virus (50–100 PFU/cell) and incubated in medium 199 with actinomycin D (2 μg/ml) and 5% bovine serum at 37 °C for various intervals. Thereafter they were cooled, scraped from the glass, allowed to swell for 15 min in a hypotonic buffer (*Tris* HCl 0.01 *M* pH 7.2, EDTA 10^{-3} *M*) and disrupted in a Dounce homogenizer. The nuclei and cell debris were removed by centrifugation at 800 *g* for 10 min, the MM fraction was sedimented at 15,000 *g* for 20 min, resuspended in TMM buffer (*Tris* HCl 0.01 *M* pH 7.5; MgCl 0.001 *M*; 2 mercaptoethanol 0.01 *M*) at the concentration of 5 mg/ml of protein and stored at − 20°.

Two polymerase systems were used:

1. Nucleoside-triphosphate medium: 1 ml of polymerase preparation was resuspended in 3 ml (final volume) of incubation mixture which contained 20 μmole *Tris* HCl pH 8.0; 2 μmole $MgCl_2$; 6 μg actinomycin D; 7 μmole 2-mercaptoethanol; 0.5 μmole phosphoenolpyruvate; 0.01 mg pyruvate kinase; 0.5 μmole ATP, CTP, UTP each; 25 μCi ^{3}H-GTP (specific activity 0.6 mCi/m mole). The mixture was incubated at 37 °C, aliquots (0.3 ml) were taken at various intervals and reaction was stopped by addition of ice-cooled $HClO_4$ 0.5 *N* and $Na_2P_2O_7$ 0.1 *M*. 0.1% caseine and 10% trichloracetic acid (TCA) was added, TCA precipitable material was sedimented on Millipore filters, washed with 5% TCA, dried with alcohol and the filters were placed into vials with toluene (PPO + POPOP) scintillator for counting radioactivity in a Packard-Tricarb liquid scintillation counter.

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2. Medium 199: 1 ml of polymerase preparation was resuspended in 3 ml (final volume) of medium 199 with actinomycin D (6 μg) and ^{3}H uridine (25 μCi, specific activity 23 mC/m mole) and incubated at 37 °C. Aliquots (0.3 ml) were taken at various intervals and treated as above.

For gradient analysis of RNA synthesized the polymerase systems after incubation were treated with 1% sodium dodecyl sulfate (final concentration) for 4 min, then ice-cooled KCl 0.1 *M* was added and the mixture vigorously shaken with the equal volume of water-saturated phenol. Phenol extraction was repeated twice,

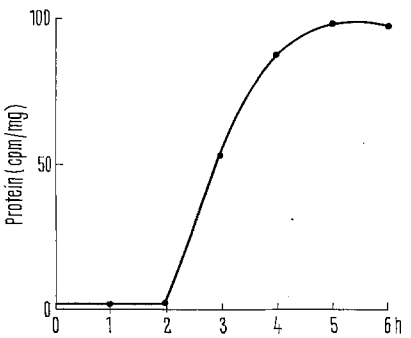

Fig. 1. Polymerase activity in chick embryo fibroblasts infected with VEE virus at various intervals after infection. The maximum of ^{3}H-GTP incorporation into TCA-precipitable material is taken as 100%. The MM fraction from virus-infected cells was incubated for 60 min by method 1.

RNA in the water phase was collected, sedimented with 2 volumes of ethanol with addition of 1.6% sodium acetate and stored at — 20 °C overnight. The precipitate freed from ethanol was dissolved in *Tris* buffer (*Tris* HCl 0.01 *M* pH 7.2; NaCl 0.1 *M*; EDTA 10^{-3} *M*), layered on a linear sucrose density gradient 5–20% and centrifuged in a bucket rotor 3 × 20 of a MSE superspeed 50 centrifuge at 20,000 *g* for 16 h. The gradients were fractionated in a LKB collector with a uvicord spectrophotometer (A_{258}) and the gradient fractions were treated as above.

Figure 1 presents the time-course of polymerase activity assayed by method 1. It is seen that virus-specific polymerase appears at 3 h after infection, its activity thereafter increases and reaches a plateau by 5 h after infection. In further experiments the MM fraction was isolated from virus-infected cells 4 h after infection.

A comparison of 2 systems for the assay of polymerase activity is shown in Figure 2. It is seen that the synthesis of RNA in nucleoside-triphosphate medium reaches a plateau within 30–45 min and thereafter no essential increase of RNA synthesis takes place. In contrast, RNA synthesis in medium 199 continues up to 3 h gradually reaching a maximum by that time. The amount of RNA synthesized in medium 199 is 40-fold as compared with nucleoside triphosphate medium.

Gradient analysis of RNA synthesized in both systems is shown in Figure 3. There is similarity of sedimentation pattern of RNA synthesized in nucleoside triphosphate medium and in medium 199. A rapidly sedimented RNA, with the sedimentation coefficient of about 40S, is ribonuclease-sensitive and presents virion RNA while RNA with the sedimentation coefficient of about 26S is partly

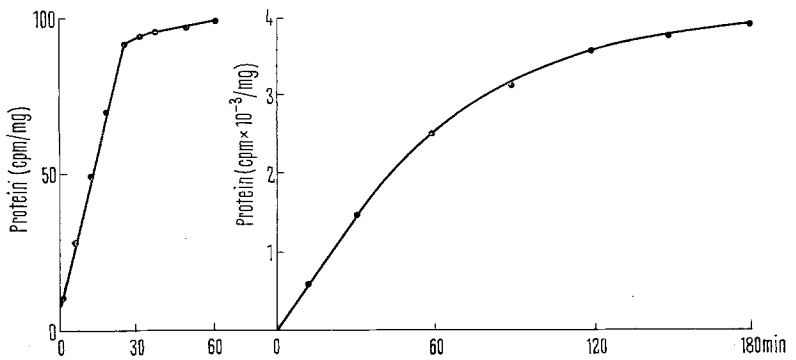

Fig. 2. Time-course of incorporation of ^{3}H-GTP into TCA precipitable material of nucleoside triphosphate medium (A) and of ^{3}H-uridine in that of medium 199 (B).

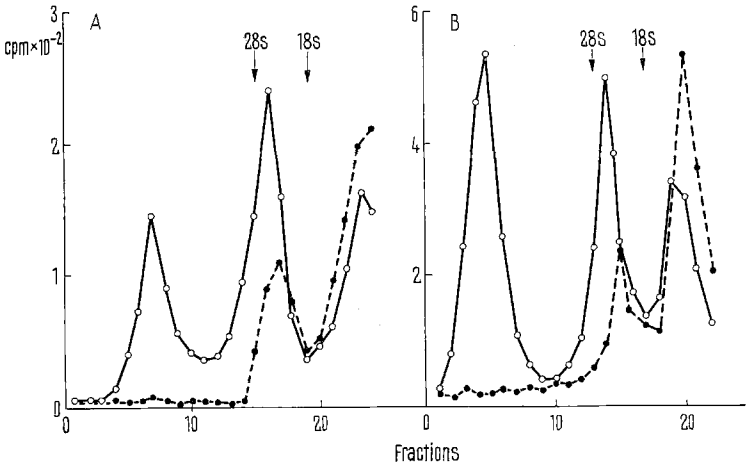

Figure 3. Distribution of ^{3}H radioactivity in gradient fractions after centrifugation in sucrose density gradients 5–20% at 20,000*g* for 16 h of RNA extracted from nucleoside-triphosphate medium (A) and medium 199 (B). The MM fraction from virus infected cells was incubated with ^{3}H-GTP for 60 min (A) and with ^{3}H uridine for 3 h (B). Solid line-total counts, dotted line-gradient fractions treated with pancreatic ribonuclease (2 μg/ml).

resistant to the enzyme can be considered as replicative intermediate of the virus. One can note, however, that both absolute and relative amounts of virion RNA are higher in medium 199 than in nucleoside triphosphate medium.

Thus, the second system for the assay of polymerase activity (medium 199) has some advantages as compared with the first system (nucleoside triphosphate medium). That may depend on the fact that RNA precursors in medium 199 (purine and pyrimidine bases) are gradually phosphorylated by mitochondria and therefore RNA synthesis lasts for several hours without inhibition of the enzyme and the templates. It may also depend on continuing protein synthesis which takes place in this coupled system.

Выводы. Были сравнены две бесклеточные системы для испытания полимеразы вируса венесуэльского энцефаломиелита лошадей, находящейся в митохондриально-микросомной фракции зараженных клеток: классическая полимеразная смесь с нуклеозид-трифосфатами и среда №199.

Вторая система имеет преимущества перед первой, так как обеспечивает продолжительный (до 3-х часов) синтез РНК, более высокий уровень синтеза и образование значительных количеств вирионной РНК.

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Effects of Unmixed and Mixed Leaf Litter of Three Species of Plants on the Development and Growth of *Polydesmus angustus* Latzel

It is well known that millipedes together with other soil borne organisms help breaking down the leaf litter, but it is not known if they themselves are affected in the process. Little has also been reported on the effects of environmental conditions, including the nature of available food, on the biotic potentialities and development of diplopods. It is however known that some species of millipedes show marked preference for the leaves of some plants[1], which is correlated to the calcium contents of the leaves[2]. A significant difference in the development of millipedes grown on F_1 and F_0 layers of beech forest has also been shown[3]. The present note discusses the role of unmixed and mixed litter of 3 species of plants, under 2 environmental conditions, on the development of *Polydesmus angustus* Latzel, a common millipede in British woodlands.

Freshly emerged first instar larvae were maintained in laboratory in receptacles containing fallen and fairly rotted leaves of oak (*Quercus robur* L.), birch (*Betula verrucosa* Ehrhart) and beech (*Fagus sylvatica* L.). Larval populations were also maintained on mixed litter of oak + birch, oak + beech and birch + beech. Replicated series of each culture were kept separately in a) a constant environment with a temperature of 23 °C, constant darkness and 90% ± 5 relative humidity (RH) and b) a fluctuating environment with varying temperature (between freezing point to 30 °C), natural light and relative humidity in different hours and seasons. Because of the high feeding rates of different larval instars the leaf litter supplied had to be replenished at frequent intervals. The time for changing the food did however not coincide for all series.

The first instar larvae were reared up to the adult stage and the time taken to complete the cycle under each treatment is shown in Table I. The data suggest development was quickest when the larvae were reared on unmixed leaf litter of any plant species and maintained in a constant environment. Mixed litter of any 2 plant species in a fluctuating environment prolonged the development period. The order of development under different treatments was: unmixed litter + constant environment < unmixed litter + fluctuating environment < mixed litter + constant environment < mixed litter + fluctuating environment.

Apart from affecting the developmental rate, the litter supplied also influenced the growth of the adults. Under the constant environment, unmixed litter was conducive to the development of large adults but mixed litter resulted in the development of smaller adults (Table II).

Although these studies did not indicate how and which component(s) of the various combinations of leaf litter and environmental combinations affected the growth and development of *Polydesmus angustus*, some general inferences can be drawn. It may be that in an ecosystem with mixed stands of plants of different species the millipede will have an extended life cycle compared to an area characterized with single plant species. The differential growth rates and the development of large or small adults are also of significance in the bioenergetics of an ecosystem: shortened life cycle means not only the production of more adults in an unit time, but the development of large sized adults could lead to an increased biomass.

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Table I. Duration of development of *Polydesmus angustus* Latzel from first instar to adult stage under different combinations of leaf litter and environmental conditions

Mean time ± S.E. (in days) taken to complete the cycle from first instar to adult stage

Environment (type of litter)	Oak	Birch	Beech	Oak + Birch	Oak + Beech	Birch + Beech
Constant temperature of 23 °C and constant darkness	113 ± 23	145 ± 23	140 ± 37	223 ± 27	235 ± 21	240 ± 30
Fluctuating temperature and light	199 ± 18	211 ± 22	205 ± 27	245 ± 18	242 ± 20	250 ± 24

Figures represent mean of 4 replicates.