***In vitro* rearing of the larval endoparasitoid, *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae)**

IV. Effects of osmotic pressure and pH on larval development

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(Received 15 February 2000; Accepted 14 April 2000)

Abstract

In vitro rearing of *V. canescens* embryos and larvae was studied. Because physical requirements as well as nutritional requirements are critical for *in vitro* rearing of parasitoids, we examined the effects of osmotic pressure and pH of culture medium on larval growth and development of this parasitoid. Larvae grew in *Galleria* pupal extract (GPE) medium with osmotic pressure ranging from approximately 300 to 700 mOsmol/kg, but their growth was retarded at above 427 mOsmol/kg. *V. canescens* larvae were tolerant to a pH range of the medium from 6.1 to 7.9, and relatively rapid growth was achieved at pH ranging from 6.5 to 7.1. In this rearing system, last stadium larvae became abnormally large in body size apparently due to uptake of too much water from the medium. After cocoon formation, they excreted a watery excrement, which could partly be responsible for their failure to pupate.

Key words: *Venturia canescens*, larval endoparasitoid, *in vitro* rearing, osmotic pressure, pH

INTRODUCTION

In the field of biological control using parasitoids, one of the greatest needs is to produce adult wasps and flies at a low production cost (Simmonds, 1944; Grenier et al., 1994). Since Simmonds (1944) suggested that parasitoids might be reared on an artificial medium, *in vitro* rearing techniques have drawn the attention of many investigators. Thus far, more than 40 species of parasitoids have been successfully reared *in vitro* from embryo to adult, but these species are restricted to dipterous species and idiobiotic hymenoptera. Koinobiotic hymenoptera including egg-larval, larval, and larval-pupal endoparasitoids (Askew and Shaw, 1986) have not been successfully reared (Grenier et al., 1994; Vinson, 1994; Thompson, 1999).

Venturia canescens (Hymenoptera, Ichneumonidae) is a larval endoparasitoid of pyralid stored-products pests (Salt, 1976) such as the Mediterranean flour moth, *Ephestia kuehniella* (Lepidoptera, Pyralidae). In our previous

study, *V. canescens* was successfully reared from embryo to final stadium larva in a medium containing pupal extract of its host, *Galleria mellonella* (Lepidoptera, Pyralidae), but only a few pupae and no adults were obtained (Nakahara et al., 1997). Later, nutritional improvement was attempted, but without success.

Besides nutritional requirements, physical requirements are very important for *in vitro* rearing of endoparasitoids. Vinson (1994) stated that “in order to develop effective *in vitro* rearing programs, efforts must not only be directed to the development of nutritious artificial media in which to grow the immatures, but the other biological and physical factors needed to produce large numbers of adults must be considered.” The physical factors, such as osmotic pressure and pH of culture media are critical for parasitoid larvae (Grenier et al., 1994; Vinson, 1994). However, available information on the role of these factors is limited.

In this study, we examined the effects of osmotic pressure and pH of culture medium on *in*

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vitro development of *V. canescens* larvae. The information presented here may contribute to future studies of parasitoid biology.

MATERIALS AND METHODS

Insects. The parasitoid, *V. canescens* was maintained on final stadium larvae of *E. kuehniella* reared on whole wheat flour under a 16L-8D photoregime at 25°C (Ohbayashi et al., 1994; Nakahara et al., 1997). *G. mellonella* was reared on a semi-artificial diet according to Wani et al. (1994) at 25°C in darkness.

Basic medium. The *Galleria* pupal extract medium (GPE medium) used as a basic medium for artificial rearing of *V. canescens* (Nakahara et al., 1997) was prepared as follows. *G. mellonella* pupae (1-day-old) were heat-treated in a water bath at 60°C for 10 min, and gently squashed in MGM-450 medium (Mitsuhashi and Inoue, 1988) from which a nutritively inert substance of PVP® K-90 was omitted. The amount of the MGM-450 medium used was equal to the volume of pupae. The pupal extract collected in a centrifugation tube was centrifuged at $18,000 \times g$ for 10 min. The supernatant was filtrated (0.45 µm pore size) and stored at -40°C. When used, it was first thawed at room temperature, and then sterilized by filtration (0.22 µm pore size). The osmotic pressure of the GPE medium (350–380 mOsmol/kg) was measured by a Digital Micro Osmometer® OM 801 (Vogel, German). To raise the osmotic pressure of the medium, 0–40 mg of mannitol (Wako Pure Chemicals Industries, Ltd., Osaka, Japan) was added to 1 ml of GPE medium. When Carlson's solution (Carlson, 1946) was used for an extraction instead of MGM-450 medium, the osmotic pressure of the medium was approximately 300 mOsmol/kg. The pH of GPE medium, which was usually 6.6–6.8, was adjusted to acid or alkaline condition with 20 µl of HCl or NaOH solution (0–1.0 N).

In vitro experiment. *V. canescens* embryos (48 h post-oviposition) aseptically collected were individually incubated in wells of a 96-well microplate (Falcon® 3072, Becton Dickinson, NJ) with 30 µl of GPE medium according to Nakahara et al. (1997). The culture vessel was sealed with vinyl tape, and maintained in a

humidified container under a 16L-8D photoregime at 25°C. The larval stadium was determined by counting the number of exuviae left in the culture medium under a dissecting microscope. The larvae that shed a head capsule and thoracic cuticle were regarded as larvae that had undergone an ecdysis. The parasitoids were checked daily for a 40-day period.

Final stadium larvae left in culture medium continued feeding and failed to pupate in previous studies (Nakahara et al., 1997; Yamamoto et al., 1997). Such larvae could be rescued from the culture medium by removing the excessive medium. The medium was removed completely from the wells containing a mature larva 0.5, 1.0, 1.5, and 2.0 days after ecdysis to the final larval stadium, and then each well was plugged with a filter-paper disc and absorbent cotton according to Nakahara et al. (1997).

Parasitoid growth. In another experiment, the fastest growth of *V. canescens* was obtained at 29°C, although the yield of final stadium larvae did not differ from that at 25°C (Nakahara and Iwabuchi, 2000). Thus, *in vitro* growth of *V. canescens* was examined at 29°C. Ten dozen 3-h-old embryos were cultured as above, and the culture medium was replenished daily from day 6 onward. Larvae which showed marked growth retardation were discarded. Ten embryos were selected and their size was measured under a phase-contrast microscope. The volume of an embryo was calculated by a formula:

$$V = LW \times SW^2 \times \pi / 6$$

where V = volume of embryo, LW = long width, and SW = short width of embryo. From day 3 onward, five larvae were taken out and anesthetized on ice, and their body length was measured under a dissecting microscope. Larvae were weighed according to Sequeira and Mackauer (1992). The 4th or 5th stadium larvae were individually put on a preweighed aluminum pan, and weighed by an electronic microbalance. Then, each parasitoid larva was dried in an oven at 100°C for 48 h and weighed again. Water content of the body was calculated by the formula:

Water content (%) = $(WW - DW) / WW \times 100$
where WW = wet weight, DW = dry weight of larva.

In vivo experiment. To examine the growth of *V. canescens* in vivo, last stadium larvae of the host were stung once by *V. canescens*. Oviposition was confirmed by the parasitoid's unique behavior referred to as "cooking" (Rogers, 1972). The parasitized hosts were individually reared for various lengths of time on whole wheat flour in plastic Petri dishes (35 mm dia, Falcon® 1008, Becton Dickinson, NJ) under a 16L-8D photoregime at 29°C, and dissected in Carlson's solution to determine the body size and weighed of the parasitoid embryos or larvae inside, as described above.

Data analysis. The percentage of hatched larvae was calculated based on the number of embryos set on culture, and the percentage of larvae molting to each stadium based on the number of hatched larvae. Data for developmental periods were analyzed by ANOVA and least significant difference (LSD) test.

RESULTS

Effect of osmotic pressure of medium

Cultured *V. canescens* larvae attained the final (5th) larval stadium when the osmotic pressure of GPE medium ranged from 296 to 698 mOsmol/kg (Fig. 1). However, as the osmotic pressure increased, they took longer to develop to the 2nd or 5th stadium (Fig. 2). At osmotic pressure of over 427 mOsmol/kg, the developmental period from embryo to the 5th stadium was significantly longer than that observed in an unmodified GPE medium (365 mOsmol/kg) (ANOVA, $p < 0.05$). No embryos hatched at more than 816 mOsmol/kg.

Effect of medium pH

Last stadium larvae of *V. canescens* were obtained in GPE medium with the pH ranging from 6.1 to 7.9 (Fig. 3). However, the developmental period from embryo to the 5th stadium was significantly prolonged when the pH was lower than 6.4 or higher than 7.2 (Fig. 4, ANOVA, $p < 0.05$). No hatching occurred when the pH was lower than 5.8 or higher than 8.4. It was noted that pH-adjustment to these values led to the precipitation of some substances. Thus, GPE medium was adjusted to near pH 5.5 or 8.0 by an addition of HCl and NaOH, respectively, and the precipitates were removed

Fig. 1. Yields of hatchlings (a), 2nd (b), and 5th stadium larvae (c) of *V. canescens* in GPE medium with increased osmotic pressure at 25°C. The culture was started with 48-h-old embryos. The percentage of hatched larvae was calculated based on the number of embryos set on the culture, and the percentage of larvae molting to each stadium based on the number of hatched larvae.

by centrifugation, followed by neutralization with NaOH or HCl. In the neutralized medium which was adjusted once to acid or alkaline condition, *V. canescens* developed to the last larval stadium, and the developmental period was not significantly different from that of the

Fig. 2. Developmental period of *V. canescens* required to reach the 2nd (closed circle) and 5th larval stadium (closed diamond) in GPE medium with increased osmotic pressure at 25°C (mean $\pm$ SD). The culture was started with 48-h-old embryos.

Fig. 3. Yields of hatchlings (a), 2nd (b), and 5th stadium larvae (c) of *V. canescens* in GPE medium with variously adjusted pH. The culture was started with 48-h-old embryos. The percentage of hatched larvae was calculated based on the number of embryos set on the culture, and the percentage of larvae molting to each stadium based on the number of hatched larvae.

larvae reared in the unmodified GPE medium (data not shown). This result indicated that the poor growth under acid and alkaline conditions was not caused by a reduction in nutritional quality of the medium.

Fig. 4. Developmental period of *V. canescens* required to reach the 2nd (closed circle) and 5th larval stadium (closed diamond) in GPE medium with variously adjusted pH (mean $\pm$ SD). The culture was started with 48-h-old embryos.

Growth and development of *V. canescens*

In vivo and *in vitro* growth of *V. canescens* embryos is shown in Fig. 5. In both cases, embryos increased in size gradually, reached a maximum at the segmented stage, and then reduced their size before hatching. Embryo growth was slightly slower *in vitro* compared to *in vivo*, though the difference occurred mainly before the blastoderm stage.

Larval growth is shown in Fig. 6. *In vitro* cultured larvae underwent ecdysis to the 2nd stadium on day 8, 3 days later than the time of appearance of 2nd stadium larvae *in vivo*. Then, they grew rapidly and eventually reached a size much larger than the maximum size of larvae grown *in vivo*.

To induce a series of actions related to metamorphosis, cultured larvae were rescued from the culture medium 0.5, 1.0, 1.5, and 2.0 days after ecdysis to the last larval stadium. Irrespective of the time of rescue, most larvae formed a cocoon in which they excreted a very watery excrement, and reached the prepupal stage. The excreted material remained on the posterior part of the larvae, which might have been partly responsible for the failure of pupa-

Fig. 5. Embryonic growth of *V. canescens* in GPE medium (closed circle) and in a host, *E. kuehniella* (open circle) at 29°C (mean $\pm$ SD). *In vitro* culture was started with 3-h-old embryos. Embryonic volume was calculated by a formula: $V = LW \times SW^2 \times \pi/6$. Capital letters with arrows (closed: *in vitro*, open: *in vivo*) indicate embryonic stage; B: blastoderm stage; G: germband stage; S: segmented stage; H: hatching.

Fig. 6. Larval growth of *V. canescens* in GPE medium (closed circle) and in a host, *E. kuehniella* (open circle) at 29°C (mean $\pm$ SD). *In vitro* culture was started with 3-h-old embryos, and the culture medium was replenished daily from day 8. Capital letters with arrows (closed: *in vitro*, open: *in vivo*) indicate ecdysis of the larvae.

tion in most individuals.

Water contents of *V. canescens* larvae

The wet weight of cultured 4th and 5th stadium larvae was 1.5–2 fold heavier than that of the *in vivo* counterparts. Conversely, the dry weight was smaller in the former than in the

Fig. 7. Wet (diamond) and dry (circle) body weight of 4th and 5th stadium larvae of *V. canescens* grown in GPE medium (closed symbols) and in a host, *E. kuehniella* (open symbols) at 29°C (mean $\pm$ SD). Day 0 is the time of ecdysis to the 5th larval stadium. Capital letters on open diamonds indicate developmental stage; E: emergence; S: spinning; C: cocoon formation; PP: prepupa; P: pupa.

Fig. 8. Water content of 4th and 5th stadium larvae of *V. canescens* grown in GPE medium (closed bar) and in a host, *E. kuehniella* (open bar) at 29°C (mean $\pm$ SD).

latter (Fig. 7). As illustrated in Fig. 8, the water content was significantly higher for *in vitro* cultured larvae (80–90%) than for the *in vivo* counterparts (50–70%; ANOVA, $p < 0.01$).

DISCUSSION

Effect of rearing conditions

For the practice of *in vitro* rearing of parasitoids, physical requirements should be considered as well as nutritional requirements. Parasitoid larvae may experience much stress under *in vitro* rearing conditions, which are often different from natural conditions in various ways. In order to reduce such stress, it might be best to prepare the culture medium for parasitoids by imitating the host haemolymph. In this paper, we focused on the role of physical conditions of the culture medium for *V. canescens*.

Both the osmotic pressure and the pH of culture medium are likely to affect survival and growth of parasitoids greatly. In general, optimal growth of cultured larvae is attained in media with an osmolarity ranging from 300 to 450 mOsmol/kg, which roughly corresponds to the range for the haemolymph of lepidopterous larvae (Thompson, 1986; Grenier et al., 1994; Vinson, 1994), although some parasitoids are tolerant to osmotic pressure as high as 1,700 mOsmol/kg (Yazgan, 1972; Thompson, 1975). In *Pachycrepoideus vindemiae* (Hymenoptera, Pteromalidae), larvae are tolerant to relatively high osmotic pressure approaching 700 mOsmol/kg (Thompson, 1981), but no adults can be

obtained unless the osmotic pressure of the medium is reduced to approximately 400 mOsmol/kg (Thompson et al., 1983). Likewise, *V. canescens* larvae are tolerant to 700 mOsmol/kg, but the medium with osmolarity under 427 mOsmol/kg is suitable.

Few studies have approached the effect of pH of the culture medium on parasitoid growth. *Eucelatoria bryani* (Diptera, Tachinidae) is highly tolerant to a wide range of pH (5.5–8.0), though a neutral condition appears to be more suitable for this species (Nettles, 1986). On the other hand, tolerance of *Trichogramma confusum* (Hymenoptera, Trichogrammatidae) is restricted in a narrow range (pH 6.7–6.95) (Grenier et al., 1994). The pH of insect haemolymph generally ranged from 6.4 to 6.8 (Mullins, 1985). Most artificial media for parasitoids have a pH near 6.8 (Grenier et al., 1994). The GPE medium used in this study also shows a pH around 6.8. *V. canescens* could develop to the last larval stadium when the pH of the medium was between 6.1 and 7.9 and rapid growth was observed with a range of pH between 6.4 and 7.2. This result was similar to the case of *E. bryani* (Nettles, 1986).

Growth and development of *V. canescens*

Development of *in vitro* embryos was slightly delayed during the initial phase of culture compared to the *in vivo* counterparts. This delay might have been due to some effect of handling at transfer to the culture medium. After the blastoderm stage, however, growth rate was almost identical in the two groups. This may indicate that GPE medium was suitable for the culture of *V. canescens* embryos. On the other hand, larval growth was much delayed in this medium compared to the *in vivo* counterparts. This may suggest that the medium was poor in quality or/and quantity for larval growth of this species.

Although GPE medium supports larval development to the last larval stadium, few larvae attain the pupal stage in *V. canescens* (Nakahara et al., 1997). Yamamoto et al. (1997) reported that successful pupation was obtained in the same species when 4th stadium larvae removed from the hosts were cultured. They used a 1:1 mixture of MGM-450 medium (10%

FBS) and SKM medium, a medium developed for an entomogenous nematode (Ogura and Miyama, 1989), and transferred the mature larvae to a pupation chamber 2 days after ecdysis to the last larval stadium. In the present study, however, no pupae were obtained (data not shown), when 4th stadium larvae cultured from the embryonic stage in GPE medium were handled according to Yamamoto et al. (1997). It is noted, however, that the larvae cultured in GPE medium grew much faster during the 4th stadium than those in the medium used by Yamamoto et al. (1997). Thus, the former medium is more suitable for *in vitro* culture of *V. canescens* than the latter.

In vitro cultured larvae reached a much larger body size than the *in vivo* counterparts in the present study. This difference might be attributed to overfeeding in the former. Overfeeding is often reported in other *in vitro* rearing systems (Hoffman et al. 1975; Strand and Vinson, 1985; Strand et al., 1988). In some cases, the problem was solved by precise-timing rescue of larvae from the culture medium (Strand and Vinson, 1985; Strand et al., 1988). In our experiment, the situation was not improved, no matter when the rescue was done. Although most larvae formed a cocoon and reached the prepupal stage, few pupae were obtained. One possible factor responsible for this failure might be the watery excrement stuck to their body.

Because the mid-gut of parasitoid larvae is blind, unassimilated materials are excreted all at once before pupation. It was found that the larvae cultured in GPE medium contained an unusually large amount of water, which made the excrement very watery. This situation is similar to the case in a pupal parasitoid, *Brachymeria intermedia* (Hymenoptera, Chalcididae): cultured larvae contained more water, accumulated less protein in the fat body, and produced fewer pupae than larvae grown in the host (Thompson, 1980). Later, *B. intermedia* was reared successfully from embryo to adult on a diet containing meat homogenate prepared for human babies at the weaning period (Dindo et al., 1997). Parasitoid growth is often improved by utilization of a retainer by which the medium is formulated into a semi-solid me-

dium or a slurry (Thompson, 1975; Nettles et al., 1980; Guerra et al., 1993; Watanabe and Mitsuhashi, 1995; Dindo et al., 1997). In a preliminary study, we also examined the effect of retainers, such as agar, gelatin, cellulose powder, powdered chicken egg yolk, albumin, and casein, on the growth of *V. canescens*, but none of them improved the performance of *V. canescens* markedly (unpublished).

The water that the parasitoid larvae take up from the medium should be reduced. Recently, Hu and Vinson (1998) reported an excellent method for *in vitro* rearing of a larval endoparasitoid, *Campoletis sonorensis* (Hymenoptera, Ichneumonidae), in which an agar layer under liquid culture medium absorbed water gradually from the culture medium, which provided a drier environment and improved larval growth. This method could be applied to *V. canescens*.

However, a semi-solid medium containing retainers and agar foundation is relatively hard to handle. In *V. canescens*, daily replenishment of the medium was necessary for successful cocoon formation (Nakahara et al., 1997). Unlike those parasitized by idiobionts, the hosts parasitized by koinobiontic wasps continue to feed and grow (Askew and Shaw, 1986), ensuring a constant supply of fresh nutrients to the parasitoid larvae. On the contrary, the culture medium will decline in quality as it grows older, unless it is replenished.

Although the watery excrement excreted by such individuals may not be the only cause for the failure of pupation in the present study, further improvement of the culture method for *V. canescens* should be made to resolve the problem of cultured larvae taking up too much water. It is possible that GPE medium is short of nutrients or essential component(s) such as a factor required for pupation, as suggested for *Trichogramma pretiosum* (Irie et al., 1987). However, when further nutritive substances are added to the medium, care must be taken to maintain the osmotic pressure and pH to within a suitable range for the insect.

ACKNOWLEDGEMENTS

We are grateful to Dr. Seiji Tanaka of the National Institute of Sericultural and Entomological Science for

critical reading of this manuscript. This work was supported in part by a Grant-in-Aid from the Ministry of Agriculture, Forestry and Fisheries of Japan.

REFERENCES

- Askew, R.R. and M.R. Shaw (1986) Parasitoid communities: their size, structure and development. In *Insect Parasitoids* (J. Waage and D. Greathead eds.). Academic Press, London, pp. 225–264.
- Carlson, J.G. (1946) Protoplasmic viscosity changes in different regions of the grasshopper neuroblast during mitosis. *Biol. Bull.* 90: 109–121.
- Dindo, M.L., C. Sama, P. Fanti and R. Ferneti (1997) *In vitro* rearing of the pupal parasitoid *Brachymeria intermedia* (Hym.: Chalcididae) on artificial diets with and without host components. *Entomophaga* 42: 445–453.
- Grenier, S., P.D. Greany and A.C. Cohen (1994) Potential for mass release of insect parasitoids and predators through development of artificial culture techniques. In *Pest Management in the Subtropics. Biological Control—a Florida Perspective* (D. Rosen, F.D. Bennett and J.L. Capinera eds.). Intercept Ltd., Andover, England, pp. 181–205.
- Guerra, A.A., K.M. Robacker and S. Martinez (1993) *In vitro* rearing of *Bracon mellitor* and *Catolaccus grandis* with artificial diets devoid of insect components. *Entomol. Exp. Appl.* 68: 303–307.
- Hoffman, J.D., C.M. Ignoffo and W.A. Dickerson (1975) *In vitro* rearing of the endoparasitic wasp, *Trichogramma pretiosum*. *Ann. Entomol. Soc. Am.* 68: 335–336.
- Hu, J.S. and S.B. Vinson (1998) The *in vitro* development from egg to prepupa of *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) in an artificial medium: importance of physical factors. *J. Insect Physiol.* 44: 455–461.
- Irie, K., Z.-N. Xie, W.C. Nettles, Jr., R.K. Morrison, A.C. Chen, G.M. Holman and S.B. Vinson (1987) The partial purification of a *Trichogramma pretiosum* pupation factor from hemolymph of *Manduca sexta*. *Insect Biochem.* 17: 269–275.
- Mitsuhashi, J. and H. Inoue (1988) Obtainment of a continuous cell line from the larval fat bodies of the mulberry tiger moth, *Spilosoma imparilis* (Lepidoptera: Arctiidae). *Appl. Entomol. Zool.* 23: 488–490.
- Mullins, D.E. (1985) Chemistry and physiology of the hemolymph. In *Comprehensive Insect Physiology Biochemistry and Pharmacology, Vol. 3* (G.A. Kerkut and L.I. Gilbert eds.). Pergamon Press, New York, pp. 355–400.
- Nakahara, Y. and K. Iwabuchi (2000) Investigation of low thermal threshold for development of the larval endoparasitoid, *Venturia canescens* (Hymenoptera: Ichneumonidae) using *in vitro* culture technique. *Entomol. Sci.* 3: 19–23.
- Nakahara, Y., K. Iwabuchi and J. Mitsuhashi (1997) *In vitro* rearing of the larval endoparasitoid, *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae). III. Growth promoting ability of extract of pupae. *Appl. Entomol. Zool.* 32: 91–99.
- Nettles, W.C., Jr. (1986) Effects of soy flour, bovine serum albumin, and three amino acid mixtures on growth and development of *Eucelatoria bryani* (Diptera: Tachinidae) reared on artificial diets. *Environ. Entomol.* 15: 1111–1115.
- Nettles, W.C., Jr., C.M. Wilson and S.W. Ziser (1980) A diet and methods for the *in vitro* rearing of the tachinid, *Eucelatoria* sp. *Ann. Entomol. Soc. Am.* 73: 180–184.
- Ogura, N. and Y. Miyama (1989) Artificial culture of an entomogenous nematode, *Steinernema kushidai* (Nematoda:

- Steinernematidae). *Appl. Entomol. Zool.* 24: 112–116.
- Ohbayashi, T., K. Iwabuchi and J. Mitsuhashi (1994) *In vitro* rearing of a larval endoparasitoid, *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae). I. Embryonic development. *Appl. Entomol. Zool.* 29: 123–126.
- Rogers, D. (1972) The ichneumon wasp *Venturia canescens* oviposition and avoidance of superparasitism. *Entomol. Exp. Appl.* 15: 190–194.
- Salt, G. (1976) The hosts of *Nemeritis canescens*, a problem in the host specificity of insect parasitoids. *Ecol. Entomol.* 1: 63–67.
- Sequeira, R. and M. Mackauer (1992) Nutritional ecology of an insect host-parasitoid association: the pea aphid-*Aphidius ervi* system. *Ecology* 73: 183–189.
- Simmonds, F. J. (1944) The propagation of insect parasites on unnatural hosts. *Bull. Entomol. Res.* 35: 219–226.
- Strand, M. R. and S. B. Vinson (1985) *In vitro* culture of *Trichogramma pretiosum* on an artificial medium. *Entomol. Exp. Appl.* 39: 203–209.
- Strand, M. R., S. B. Vinson, W. C. Nettles, Jr. and Z. N. Xie (1988) *In vitro* culture of the egg parasitoid *Telenomus heliothidis*: the role of teratocytes and medium consumption in development. *Entomol. Exp. Appl.* 46: 71–78.
- Thompson, S. N. (1975) Defined meric and horidic diets and aseptic feeding procedures for artificially rearing the ectoparasitoid *Exeristes roborator* (Fabricius). *Ann. Entomol. Soc. Am.* 68: 220–226.
- Thompson, S. N. (1980) Artificial culture techniques for rearing larvae of the chalcidoid parasite, *Brachymeria intermedia*. *Entomol. Exp. Appl.* 27: 133–143.
- Thompson, S. N. (1981) *Brachymeria lasus* and *Pachycrepoideus vindemiae*: sterol requirements during larval growth of two hymenopterous insect parasites reared *in vitro* on chemically defined media. *Exp. Parasitol.* 51: 220–235.
- Thompson, S. N. (1986) Nutrition and *in vitro* culture of insect parasitoids. *Annu. Rev. Entomol.* 31: 197–219.
- Thompson, S. N. (1999) Nutrition and culture of entomophagous insects. *Annu. Rev. Entomol.* 44: 561–592.
- Thompson, S. N., L. Bedner and H. Nadel (1983) Artificial culture of the insect parasite, *Pachycrepoideus vindemiae*. *Entomol. Exp. Appl.* 33: 121–122.
- Vinson, S. B. (1994) Parasitoid *in vitro* rearing: successes and challenges. In *Techniques of Insect Rearing for the Development of Integrated Pest and Vector Management Strategies* (J. P. R. Ocheng'-Odero ed.). ICIPE Science Press, Nairobi, Kenya, pp. 49–108.
- Wani, M., K. Iwabuchi and J. Mitsuhashi (1994) Developmental responses of *Galleria mellonella* (Lepidoptera, Pyralidae) larvae to parasitism by a braconid parasitoid, *Apanteles galleriae* (Hymenoptera, Braconidae). *Appl. Entomol. Zool.* 29: 193–201.
- Watanabe, M. and J. Mitsuhashi (1995) *In vitro* rearing of an endoparasitic fly, *Exorista sorbillans* (Diptera: Tachinidae). *Appl. Entomol. Zool.* 30: 319–325.
- Yamamoto, Y., M. Oori, T. Ohbayashi, K. Iwabuchi and J. Mitsuhashi (1997) *In vitro* rearing of a larval endoparasitoid, *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae) II. Larval development. *Appl. Entomol. Zool.* 32: 256–258.
- Yazgan, S. (1972) A chemically defined synthetic diet and larval nutritional requirements of the endoparasitoid *Itopectis conquisitor* (Hymenoptera). *J. Insect Physiol.* 18: 2123–2141.