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BIOLOGICAL CONTROL OF PESTS THROUGH EGG PARASITOIDS OF THE GENERA *TRICHOGRAMMA* AND/OR *TRICHOGRAMMATOIDEA*

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Insects of the genus *Trichogramma* are small size (less than 1mm) Hymenoptera, and egg parasitoids exclusively. They are highly specialized, having sensorial organs on antennae and legs capable of detecting eggs of several Lepidoptera species, even though they have also been cited to occur on over 200 species belonging to 70 families and 8 orders (Morrison, 1985).

In pest management programs, the *Trichogramma* species are among those which have been studied and utilized most in the world. The importance of these parasitoids is so great that there is a world group working on *Trichogramma*. These researchers meet every four years in different countries to discuss the advances made in their research.

These natural enemies have been utilized in inundative releases in several countries of the world against a large number of pests of agricultural importance (Parra & Zucchi, 1986 and Parra et al., 1987a).

In socialist countries, such as Russia, insects are reared in biofactories and then released over extensive areas. Only last year, nearly 15 million hectares were "treated" with these parasitoids, with the aim of controlling about 20 different agricultural pest species. In other countries these insects are sold. In the U.S.A. there are 23 commercial companies distributed over 10 states (Ridgway et al., 1986) and this insect is also marketed in South America (Colombia).

In Brazil the perspectives for control using this parasitoid are very favorable for several pest species, considering the abundance of *Trichogramma* occuring naturally in the field.

However, basic studies beginning with collection and identification of species and continuing until evaluation of their efficiency under field conditions are needed.

Two *Trichogramma* programs are underway in Brazil: one involving forest pests,which is being developed by the Federal University of Minas Gerais which began in 1975 (Moraes et al., 1983), and the other relating to pests of agricultural importance (Department of Entomology-ESALQ), which is the object of this report.

RESEARCH ON *TRICHOGRAMMA* DEVELOPED BY THE DEPT. OF ENTOMOLOGY OF ESALQ/USP

The aim of this project, which was initiated in 1984, is the biological control of *Diatraea saccharalis* (Fabr., 1794) on sugarcane, and *Heliothis virescens* (Fabr., 1781) and *Alabama argillacea* (Hübner, 1818) on cotton. The objective of this program is to complement the biological control of the sugarcane borer (currently utilizing larva parasitoids) and to reduce the number of insecticide applications on cottons crops. It is an extensive program for long-run development (Fig. 1). In this project three researchers and about 10 undergraduate and graduate students are involved and financial supported is being provided by FINEP, FIPEC, and FAPESP.

GENERAL RESULTS

The results obtained to date are shown in the chronogram sequence of Fig. 1.

Collecting strains – Collections have been made at different sites in Brazil, with emphasis being given to the State of São Paulo (Fig. 2).

It is important that these collections be conducted in locations showing different climatic conditions, since despite their apparent lack of specificity these parasitoids show particular behaviors under varied microclimatical conditions.

Strains identification – The taxonomy of *Trichogramma* was greatly advanced with the paper of Nagarkati & Nagaraja (1971), which showed the importance of the male genitalia for specific identification. However, in addition to morphology, at times biological (crossings) and biochemical (electrophoresis) studies are required for a perfect characterization of the species. Despite the advances made, there is still some confusion relating to the identifications

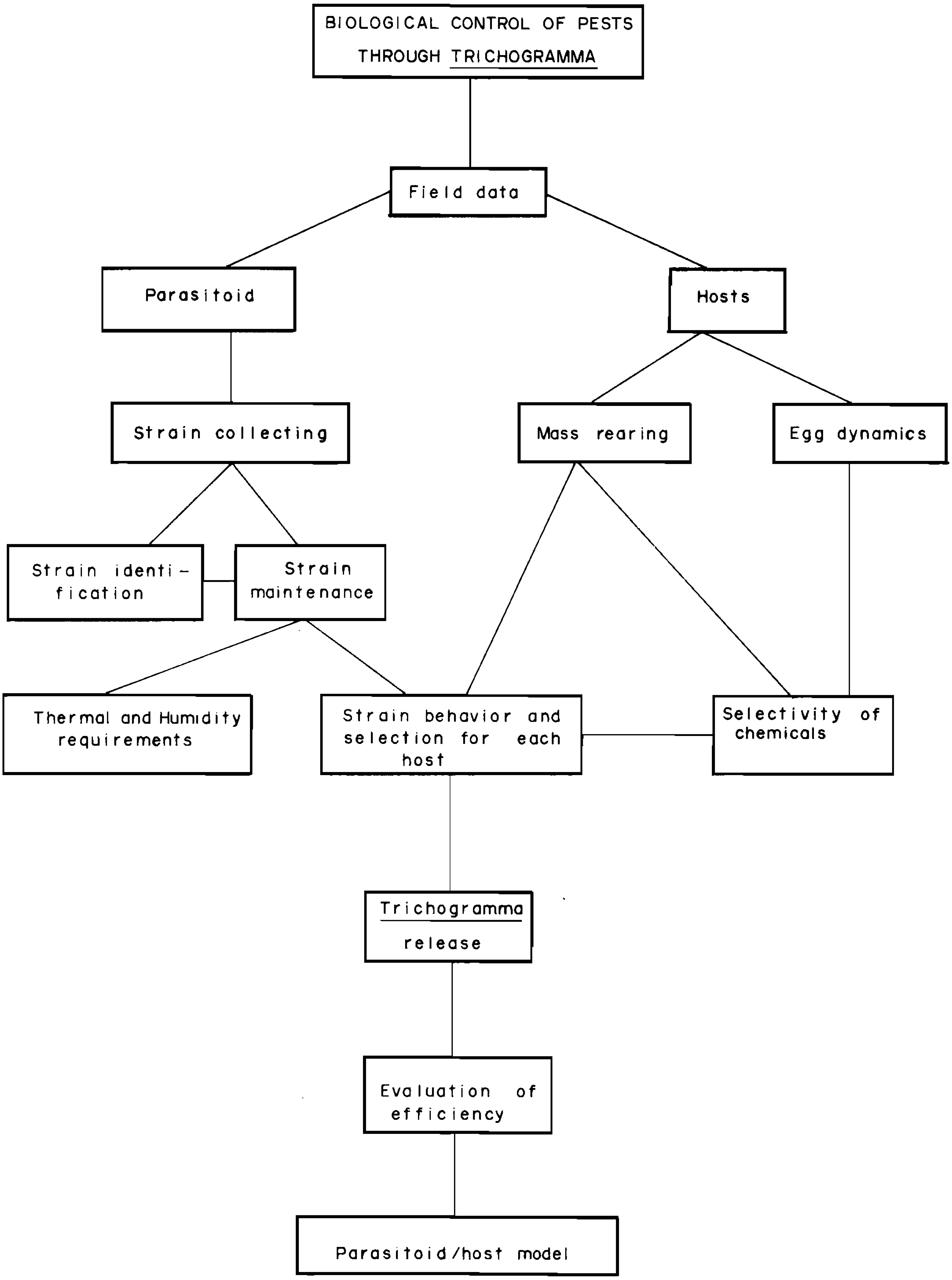

Fig. 1: Chronogram of Research Program on *Trichogramma* at the Department of Entomology – ESALQ/USP.

of *Trichogramma*, especially those conducted prior to Nagarkati & Nagaraja's work (loc. cit.). Thus, in Brazil, *T. minutum* has always been referred as the only parasitoid of the sugarcane borer. Nevertheless, Zucchi (1985), who studied hundreds of *Trichogramma* specimens obtained from sugarcane borer, never found a *T. minutum* specimen.

Based on present taxonomic knowledge, 13 *Trichogramma* species and 2 *Trichogrammatoidea* species are present in Brazil (Parra & Zucchi, 1986).

Four new *Trichogramma* species were identified from *D. saccharalis* eggs in the present project. One of the new species (*Trichogramma* sp.1), which is predominant, occurs in all of the regions studied (Fig. 2); in Junqueirópolis the species *T. pretiosum* was also found (Zucchi et al., 1987). In Alagoas and Bahia, a new species (*Trichogramma* sp.2) was always found on *D. saccharalis* eggs.

Fig. 2: Trichogramma and/or *Trichogrammatoidea* collecting locations in the State of São Paulo, on sugarcane and cotton crops.

The parasitoids collected from *H. virescens* and *A. argillacea* to date were identified as being *T. pretiosum*.

Maintaining strains – Samples are consistently collected from the field and collections having live specimens are maintained in the strain collection of the Department of Entomology, at ESALQ. Parasitoids are reared mainly on *Anagasta kuehniella* (Zeller, 1879) eggs, although in certain situations *Sitotroga cerealella* (Oliv., 1819) eggs or eggs of natutal hosts are utilized.

At present from 25 to 30 strains are maintained in the collection of the Department of Entomology, at ESALQ.

Selection of an alternative host for mass rearing of the parasitoid – In the current phase of the program, the production of host eggs is still conducted on a small scale for research purposes. *A. kuehniella*, is being reared mainly using methodology developed by Parra et al. (1985), that is, a diet consisting of 96-97% whole wheat meal and 3-4% yeast.

A new methodology intended to increase production for future releases in the field has been developed by Parra et al. (1987b). In this technique, plastic boxes (47.0 x 29.5 x 10.5 cm) containing corrugated cardboard sheets for *A. kuehniella* pupation are utilized. On these cardboard sheets, 0.45 g of eggs (approximately 16200 eggs), 1 kg of whole wheat meal and 35 g of beer yeast are placed. Soon after the emergence of the first adults, the cardboard sheets are transferred to wooden boxes (40.0 x 42.0 x 87.5 cm). This box used for collecting adults has an acrylic front wall and a funnel-shaped bottom of brass. Each box holds 12 cardboard sheets. Adults are collected dailv, after being anesthetized with CO_2 through a plastic tube adapted to the top of the box.

The adults are transferred to egg collection boxes which are made of plastic and acrylic, measuring 56.5 x 36.5 x 21 cm high, with a nylon net dividing them in two. In the upper half there is a top and a place for adults to rest. The lower part is in the form of a funnel, made of acrylic, and the eggs which pass through the nylon net are collected in a collecting drawer.

In search for a cheaper diet which will provide for a good development of the insect, comparative biological studies relating to *A. kuehniella* on different diets are being conducted based on life tables or other biological parameters.

It has been shown that there is higher egg production in the colder months of the year when room temperature are lower because there is no environmental control in the rearing room. Daily egg production is recorded on appropriate cards.

A small population of *S. cerealella* is maintained on corn kernels for comparative biological studies of the parasitoid on different alternative hosts, according to Stein's (1985) technique.

The species *Corcyra cephalonica* (Stainton, 1865), which is extensively used in China for the production of *Trichogramma*, has been maintained in the laboratory on diets consisting of ground rice (97 parts) and sugar (3 parts) or wheat germ (97 parts) and yeast (3 parts) for the purpose of evaluating, in the future, the biological behavior of *Trichogramma* species on this alternative host.

Biological and behavioral aspects of different Trichogramma species – Some results obtained to date which carry importance for the program are reported below:

- embryo death using ultra violet rays to avoid cannibalism by larvae originating from non-parasitized eggs should not be used for *D. saccharalis* eggs because this practice causes intensive drying;
- the size of the host egg affects the number of parasitoids which emerge per egg and their period of development;
- young *A. kuehniella* eggs are parasitized more intensively than those stored for several days, based on studies on *Trichogramma* sp. (collected on *D. saccharalis*) and *Trichogrammatoidea eldanae* Viggiani, 1979. There is an inverse correlation between storage period and parasitism (Lopes et al., 1987);
- regardless of host, parasitoid longevity is always longer when it has a chance to parasitize (Fig. 3);
- regardless of host presence, parasitoids that feed on pure honey live longer than those which do not;
- parasitism is more intensive on *A. kuehniella* eggs than on *S. cerealella* eggs, and on the later host there is a higher production of males;

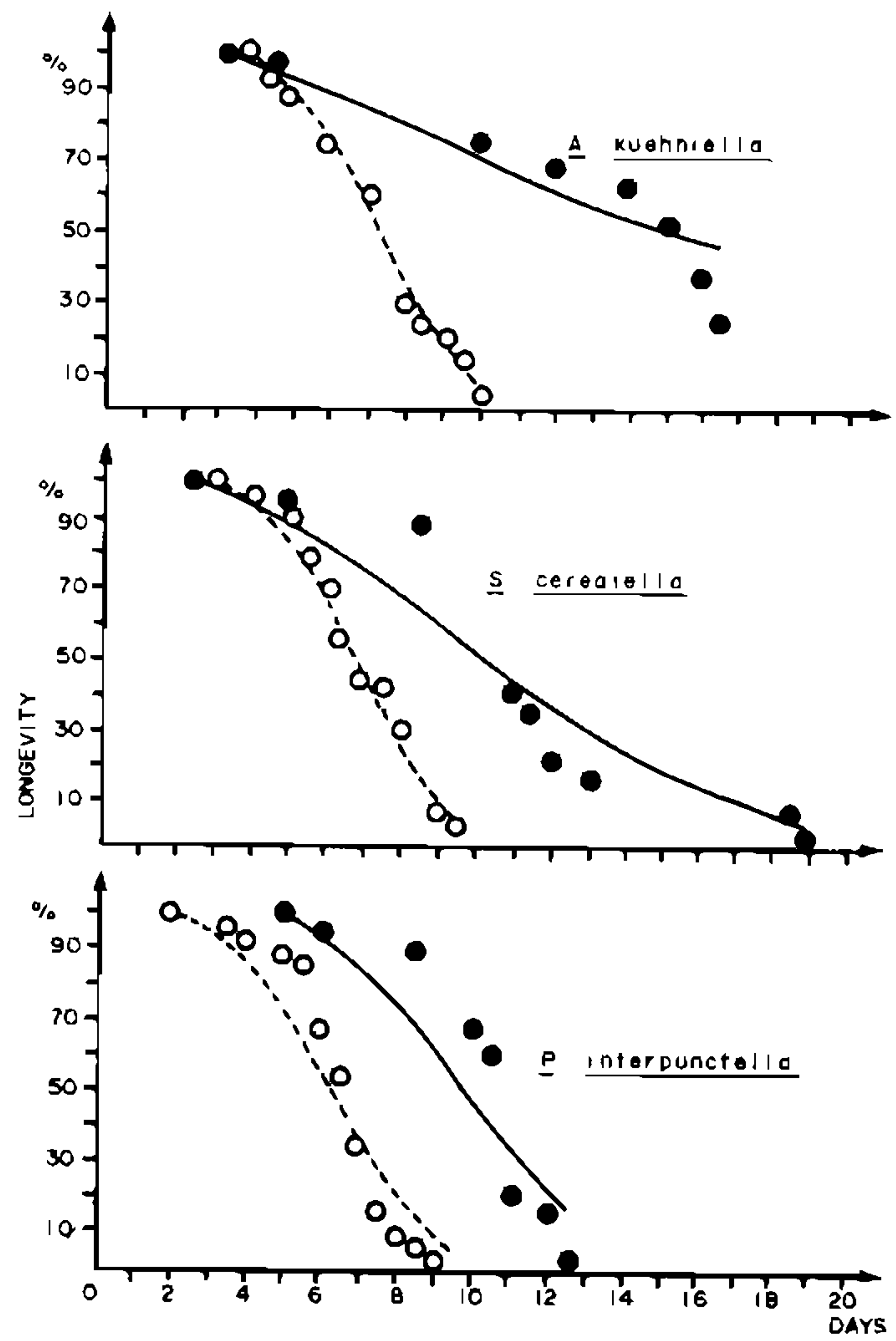

Fig. 3: Longevity curves of *Trichogramma* sp. which parasitized (————) and did not parasitize (– – – –) based on Weibull's distribution, on 3 alternative hosts (Stein & Parra, 1987a).

TABLE

Thermal requirement of *Trichogramma* species collected from *A. argillacea*, *D. saccharalis* and *H. virescens*

Species	Natural host	Host on which insect was reared	Threshold of temperature (°C)	Thermal constant (K) (degree days)
Trichogramma sp.* (pop. Piracicaba-SP)	*A. argillacea*	*A. kuehniella*	13.99	123.25
*T. pretiosum** (pop. Iguatu-CE)	" " "	" " "	12.81	133.25
*T. pretiosum** (pop. Goiânia-GO)	" " "	" " "	11.98	131.95
Trichogramma sp.$_1$**	*D. saccharalis*	" " "	11.18	163.90
Trichogramma sp.$_1$**	" " "	*S. cerealella*	11.45	177.76
Trichogramma sp.$_2$**	" " "	*A. kuehniella*	9.62	190.99
Trichogramma sp.$_2$**	" " "	*S. cerealella*	9.41	216.64
T. pretiosum (pop. Guaíra-SP)	*H. virescens*	*A. kuehniella*	13.59	108.78
T. pretiosum (pop. Guaíra-SP)	" " "	*H. virescens*	11.00	140.21

*Bleicher (1985).
**New species described by R.A. Zucchi (in press).

- *D. saccharalis* eggs, on the 3rd and 4th days of embryo development, are more intensively parasitized by *Trichogramma* sp., as compared to eggs in the first two days of development;
- the parasitism capacity of the species studied has ranged between 70 and 120 eggs per female.

Thermal and humidity requirements

Thermal requirements – The requirements of some species have been studied, and the results are shown in the Table.

The duration of the egg-adult period was affected by temperature for all strains studied. The cycle was shortened as temperature increased. The same species, when originating from distinct regions, may show a different behavior.

As a general rule, in the range of 20-22°C, there is a higher longevity of *Trichogramma* adults.

A. kuehniella has been shown to be more adequate for *Trichogramma* species than *S. cerealella* since it provides a shortening of the parasitoid cycle and a higher emergence rate (Parra et al., 1987a). Thermal requirements varied with the *Trichogramma* species. However within the same species they were the same, regardless of the host utilized (Fig. 4).

For *T. pretiosum* collected from *H. virescens* eggs, the host utilized apparently affected the physiology of the parasitoid, since the threshold of temperature of the natural host was 11°C and of the alternative host it was 13.59°C (Table), giving thermal constants of 140.21 and 108.78 degree days, respectively (Estevam et al., 1987).

Based on research by Stein & Parra (1987b), the alternative host *A. kuehniella* may have from six to seven generations in the laboratory, provided its thermal requirements are 961.12 degree days.

Humidity requirements – For rearing *Trichogramma* from *A. kuehniella* eggs, there is no need for a rigid control of the relative humidity of the air in the rearing room. Within the range of 40 to 100%, only the extremes should be avoided, as they cause a prolonging of the cycle or favor microorganism multiplication, respectively.

Thus, for *A. kuehniella*, the 60 to 90% range may be utilized, whereas for *S. cerealella* an environment with 60% RH is unfavorable and, therefore, humidities above 70% should be utilized.

It is shown that when the parasitoids are reared on natural hosts, *D. saccharalis* (especially) and *A. argillacea* eggs are very sensitive to drying, and therefore require humidities higher than 80%. *H. virescens* eggs permit the

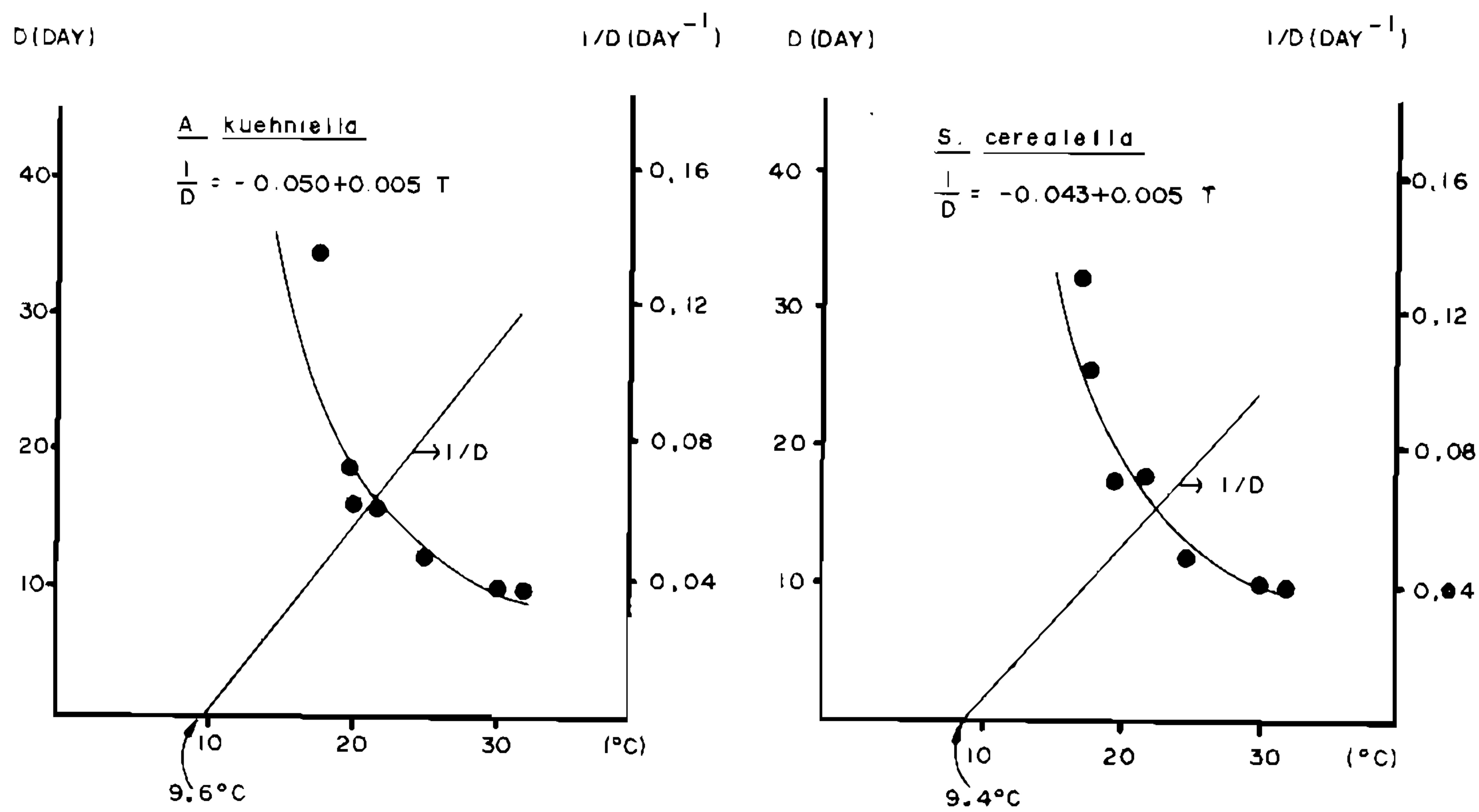

Fig. 4: Thermal requirements of *Trichogramma* sp.n. collected on *D. saccharalis* and reared on *A. kuehniella* and *S. cerealella*.

development of the parasitoid in quite low relative humidities (Almeida & Parra, 1987a, b).

Rearing natural hosts – For maintaining *D. saccharalis* and *H. virescens* colonies in the laboratory, artificial diets consiting of corn, wheat germ, and yeast are utilized for the first mentioned species (Mihsfeldt, 1985), and casein and wheat germ for the second (Moreti & Parra, 1983). In the case of *A. argillacea,* since no artifical diets are available, cotton leaves are utilized.

Dynamics of the pest eggs

D. saccharalis

Natural parasitism has reached levels of up to 80% in surveys conducted in Piracicaba, Iracemápolis, and Barra Bonita, State of São Paulo. These data may be underestimated, as the action of predators was not evaluated in these surveys, and they might have eliminated parasitized as well as non-parasitized eggs indiscriminately.

Since high RH (above 80%) is required for embryo development of *D. saccharalis* and for parasitism, it has been shown that cane varieties which grow densely in the field create a more humid microclimate which favors parasitism. Thus, parasitism varies according to different varieties; however, there is no correlation between climatical data taken macroclimatically and parasitism by *Trichogramma.*

Parasitism is always more intensive in plant-cane, which shows a higher vegetative vigor and suffers greater attack from *D. saccharalis.* In climatically normal years, parasitism is higher from October to December; in Barra Bonita it is more intensive on varieties SP-701284 and SP-7111406 and lower on variety NA-5679.

Parasitism by *Trichogramma* is highly correlated with sugarcane phenology. Thus, as a general rule, parasitism begins 5 to 7 months after sugarcane planting. On varieties which are less attacked, parasitism begins later, e. g., 7-9 months after planting.

D. saccharalis egg laying and, consequently, parasitism by *Trichogramma* are found uniformly distributed along the dorsal part of the sugarcane leaves mainly.

Higher parasitism is found on the medium and high parts of the plant. Parasitism is uniformly distributed throughout the field and it is always higher in the later generations of *D. saccharalis.*

H. virescens and *A. argillacea*

Populational levels in the region of Guaíra-SP in 1986 and 1987 (Figs. 5 and 6) show that natural parasitism is very high for both pests. In 1987, the high rainfall rate in January and February, as compared to the average for that region, probably resulted in a lower natural parasitism of *H. virescens.*

In spite of the application of chemicals of wide spectrum in the experimental area (Figs. 5 and 6), parasitism was maintained at a high levels which suggests tolerance or adaptation of the parasitoid.

Although parasitism levels were high, this occurred only in the last generations of the two pests.

Selectivity of chemicals – Laboratory tests are being conducted to determine the effect of insecticides, growth regulators and herbicides applied to sugarcane and cotton crops on the parasitoid.

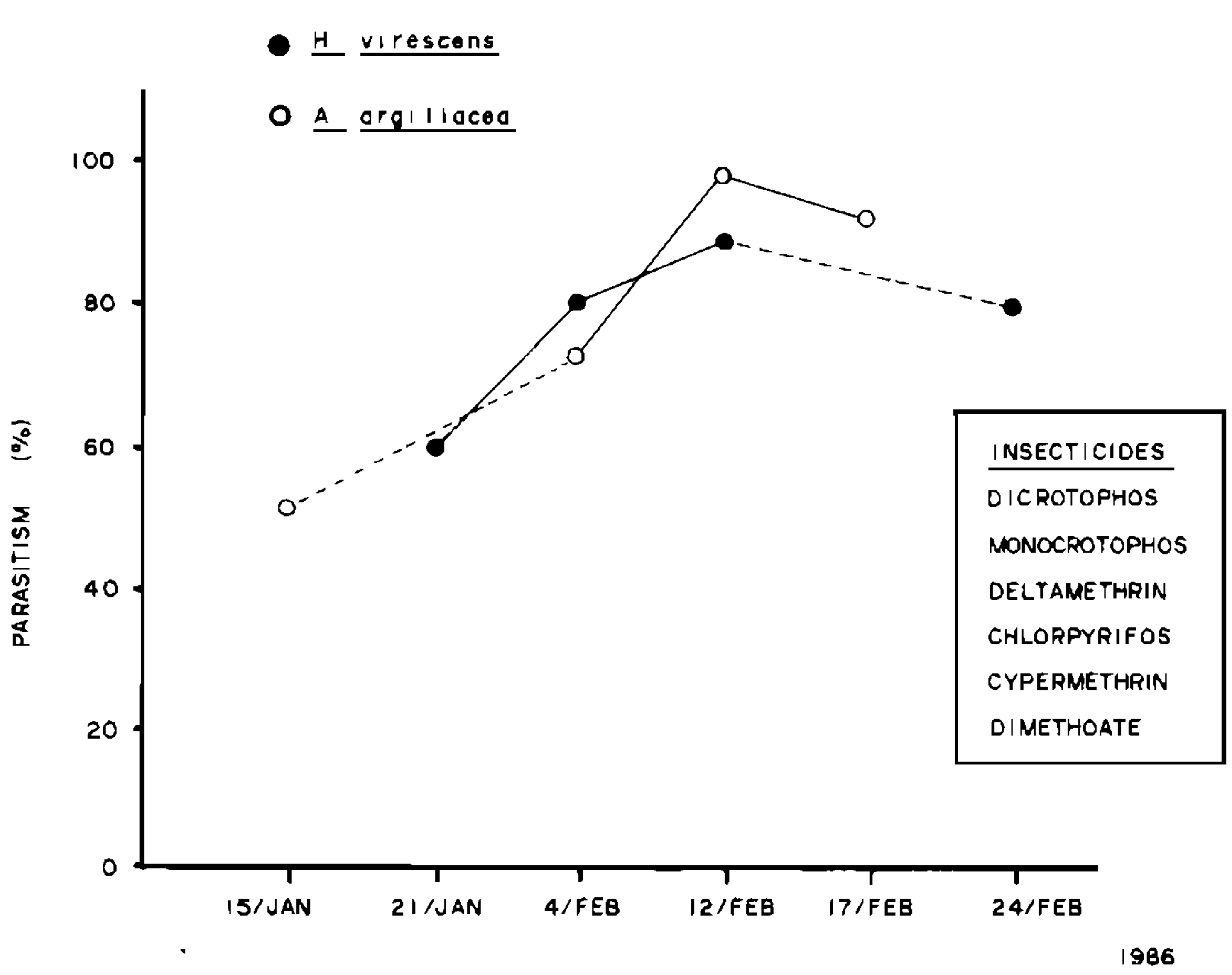

Fig. 5: Natural parasitism of *H. virescens* and *A. argillacea* by *T. pretiosum* in 1986, under the action of several insecticides. Guaíra, SP (Parra et al., 1986).

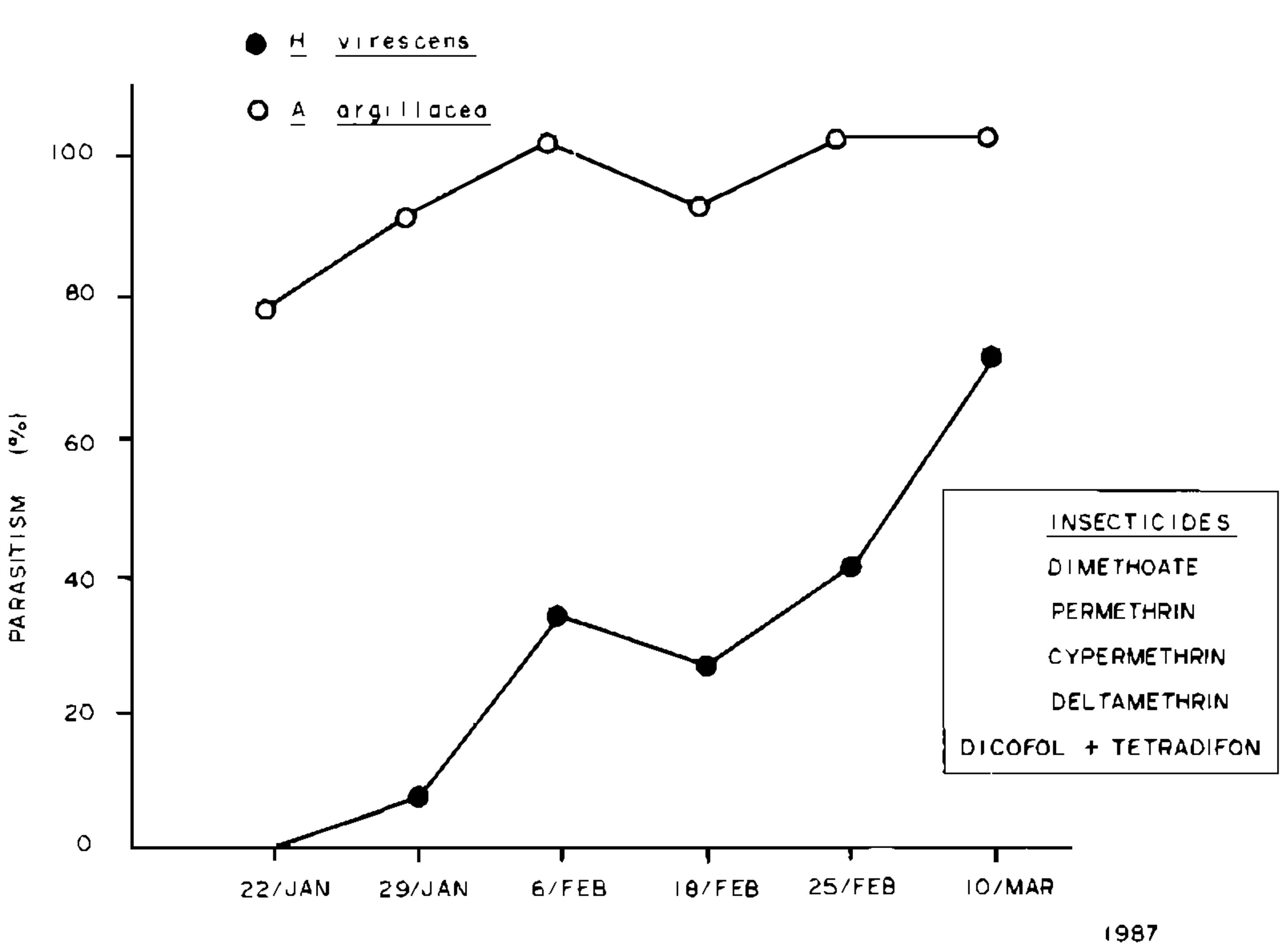

Fig. 6: Natural parasitism of *H. virescens* and *A. argillacea* by *T. pretiosum,* in 1987, under the action of various insecticides. Guaíra, SP.

In addition to the effects on *Trichogramma* parasitism and cycle also the period of time required for product degradation which will permit the release of natural enemies without damage to their population will be determined.

Parasitoid release – Tests are being carried out in greenhouses and screened cages in order to determine the numbers and dates of releases. Based on population dynamics data, the releases should be made in the first generations of the pest to avoid high pest population in later generations.

Preliminary results indicate that the number to be released is from 2 to 4 times higher than that recommended in the literature.

In view of the fact that a sex ratio of 3 to 7♀ for 1♂ of *Trichogramma* occurs under field conditions (Micheletti, 1987), the strains to be released should have, when reared in the laboratory, a sex ratio close to that found in the field, and show a parasitism capacity higher than 80%. Tests conducted on dispersion capacity of the parasitoid have shown that this dispersion is not very high. Parasitism decreases with distances more than 10m from the release point. Therefore, releases, should be made at many locations.

Another aspect worth mentioning is that for field studies with *D. saccharalis*, couples should be placed to lay on the sugarcane leaf to be studied, since when the eggs are laid on paper sheets (as commonly done in the laboratory) and taken to the field, parasitism suffers an 87% decrease.

Another area to be studied is the storage of moth eggs (parasitized or not) for the release periods. In ESALQ's *Trichogramma* laboratory the eggs are stored at 10°C for a period not longer than 20 days. Since sophisticated storage techniques which permit storage for over a year are available in other countries, studies on the thermal requirements of the egg phase of moths are being carried out to attempt to interrupt embryo development at the threshold temperature, thus permitting storage for long periods of time.

FINAL CONSIDERATIONS

The results obtained in the different phases of the program are very promising.

For *D. saccharalis*, where the egg phase is the key-phase for population growth of the pest (Botelho, 1985), the *Trichogramma* species may be another parasitoid to join the larval parasitoids currently used for controlling that insect.

On cotton, the high levels of parasitism recorded under natural conditions for *A. argillacea* and *H. virescens*, even under conditions of systematic chemical applications, demonstrate that these parasitoids are excellent control agents.

In fact, as soon as some proposed phases of the program are completed, which will permit releasing the parasitoids in adequate periods, there will be a changing of the pest/parasitoids populational curves. This will lead, in a judicious manner, to an elevation of the parasitoid populational levels in the first generations of the pests.

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