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(54) **Összeállítás, tenyésztési elrendezés és eljárás spathius exarator tenyésztésére**

COMPOSITION, BREEDING ASSEMBLY AND METHOD FOR BREEDING SPATHIUS EXARATOR

The invention relates to an arrangement for breeding *Spathius exarator*, to a breeding assembly with such an arrangement, and to a method of breeding *Spathius exarator*.

The common furniture beetle (*Anobium punctatum*) is a wood pest which is generally also known by the name woodworm. Woodworm cause great economical damage by destroying processed timber in rooms with high atmospheric humidity, especially in lofts and in churches. Active woodworm infestation can be recognized by exit holes on the wood surfaces and by mounds of bore meal formed underneath the exit holes. Various methods of controlling woodworm have been proposed in the past, for example the treatment with insecticides, the thermal treatment and also gassing with nitrogen.

A novel approach for the control of *Anobium punctatum* is the use of beneficials, that is other organisms, which are parasitoids of *Anobium punctatum*. A suitable enemy of the common furniture beetle is the braconid wasp species *Spathius exarator*, which is described as the most frequent parasitoid of *Anobium punctatum*. In order to be able to efficiently employ such a beneficial, larger, more rapidly available useable populations of *Spathius exarator* are required. However, it is not yet known from the prior art how the braconid wasp species *Spathius exarator* can be persuaded in the laboratory to oviposit, let alone how a stable mass breeding programme can be established.

The publication EP-A-1733617 discloses the control of Anobium punctatum (common furniture beetle) with Lariophagus distinguendus.

The publication US-A-3786594 discloses a method for breeding parasitic wasps with an aphid-containing host population.

AT 005240 U1 only describes a tin for parasitic wasps which allows the promotion of the parasitic wasp population in a natural parasitic wasp habitat. However, such a procedure cannot be extrapolated to a laboratory activity and offers no way of making possible the mass breeding of *Spathius exarator*.

The invention was therefore based on the object of identifying a possibility of breeding *Spathius exarator* which makes possible the mass breeding of these beneficials.

To achieve this object, there is provided in accordance with the invention an arrangement for breeding *Spathius exarator*, including

- a breeding population of *Spathius exarator*,
- a host population and
- a support for the breeding population and the host population,

which is distinguished in that

the host population displays larvae of *Stegobium paniceum* and/or *Lasioderma serricorne* and the support comprises dried baked goods, in particular bread.

The use of woodworm, that is *Anobium punctatum* itself, as has been found within the scope of the

present invention, is not suitable for the mass breeding of *Spathius exarator* since the host develops extremely slowly, and it must additionally be borne in mind that *Spathius exarator* is a parasitoid of *Anobium punctatum*, causing the death of the host. After extensive research, the inventors have found that the bread beetle (*Stegobium paniceum*) and the tobacco beetle (*Lasioderma serricorne*), which are related to *Anobium punctatum*, can be used as surrogate hosts. These beetles have the advantage that, in contrast to *Anobium punctatum*, they can be bred much more readily under laboratory conditions because they have a relatively short life cycle, which is three to four generations per year at room temperature.

However, it has also emerged that neither free bread beetle and tobacco beetle larvae nor bread beetle and tobacco beetle larvae in timber are suitable as host population due to their high mortality. Research, however, has shown that hard, old baked goods are suitable as a support for the host population and are also accepted by *Spathius exarator*. Therefore, the invention provides to make available *Stegobium paniceum* and/or *Lasioderma serricorne* as host population on a support, designed as dried baked goods, of the breeding population of *Spathius exarator*, where experiments have shown that the acceptance of the surrogate hosts by the *Spathius exarator* is extremely high and that very good reproduction rates can be achieved. Also, a surrogate reproduction environment which can be generated more rapidly and more suitably for the braconid wasps is generated in a targeted fashion.

In this manner, it is therefore possible for the first time to breed substantial amounts of *Spathius exarator* which can be employed for example as beneficials against woodworm.

It is expedient when the baked goods have been dried for at least three days, in particular in the air. In this manner, support characteristics are achieved which permit both the *Stegobium paniceum* and/or *Lasioderma serricorne* to burrow into the baked goods, in particular into the bread, but that, on the other hand, the support is also accepted by the *Spathius exarator* as a place where potential hosts can be found. Alternatively, it is also possible to dry the baked goods in particular in a baking oven for 40 to 50 minutes, for example 45 minutes, at 80°C. Preferably, the dried baked goods have a residual material moisture of between 10-13%.

It has proved to be especially advantageous when the baked goods take the form of an at least partially hollowed-out baguette. It has been found that too soft or too rough bread is less suitable since the *Spathius exarator* cannot enter with their ovipositor, or the ovipositor even gets stuck. A baguette with a fairly smooth surface has proved to be well suited. In order to prepare baguettes to act as supports, commercially available baguettes can suitably be cut open, whereafter the soft white crumb inside is preferably removed and discarded. For example, a baguette which is 60 cm in length can be divided into three portions in each case 20 cm in length, which are then divided lengthwise to form in each case two baguette sections. The baguette sections, in particular the hollowed-out baguette sections, are dried in the air for three to four days, whereupon such a dried baguette section is populated with the larvae, which, after approximately two to three days, will have burrowed below the hard crust. The *Spathius exarator* can now be added.

The remaining wall thickness of such a baguette section or generally of the baked goods can expediently

amount to from 0.8 to 1 cm. This ensures that the *Spathius exarator* can reach all burrowed larvae with their ovipositor. The resulting dish shape which results from hollowing out the baguette sections has the added advantage that the larvae which are introduced will be able to drop out less readily.

A further embodiment of the arrangement provides that the ratio of the members of the breeding population to the members of the host population is in the range of from 1:2 – 1:8, preferably 1:6. A ratio of 1:6 has proved to be especially advantageous.

Preferably, the larvae of the host population have a minimum size of 2 mm. Such larvae can be extracted from a breeding batch for example with the aid of a screen. A larvae size of 2 mm suggests that they are firstly capable of burrowing underneath the hard crust of the baked goods, in particular of the bread, and that, secondly, their size is extremely suited to the reproduction of the *Spathius exarator*.

The arrangement can expediently furthermore comprise a feed source for the *Spathius exarator*, in particular a piece of cotton treated with a honey-water solution. To safeguard the nutrition of the breeding population and/or any *Spathius exarator* which may have been bred, there is therefore provided a feed source as part of the composition, it being possible for example to add a piece of cotton with a 20% honey-water solution, which can be renewed for example every two to three days.

Besides the arrangement, the invention also relates to a breeding assembly comprising a breeding container, in particular also plastic, with an arrangement according to the invention. A particularly suitable breeding container is a faunarium which comes in different sizes and is already available on the market. If faunaria which are available on the market, or breeding containers, have unduly large ventilation gaps, for example in a lid, it can be provided for example to provide a commercially available dish cloth or other textile between the lid and the rest of the container. Everything that has been said on the arrangement according to the invention can also be applied analogously to the breeding assembly according to the invention, with which the abovementioned advantages can also be achieved. In particular, such a breeding assembly is suitable for being placed into a laboratory provided for the mass breeding of *Spathius exarator*.

The invention likewise relates to a method of breeding *Spathius exarator* using an arrangement or breeding assembly of the inventive type. Here, environmental conditions which favour a utilization of the host population by the *Spathius exarator* are therefore generated. Everything that has been said on the inventive arrangement and the inventive breeding assembly can also be applied analogously to the method according to the invention, which naturally also applies vice versa, if this is feasible. Thus, the method according to the invention can be employed in particular also for the mass breeding of *Spathius exarator*.

When the arrangement or breeding assembly is generated, one must thus first prepare the support, which, as has been described, is preferably formed of a commercially available baguette which is dried preferably for at least three, in particular to four, days. Then, the dried baguette section, or in general terms the dried baked goods, is/are first populated with larvae of *Stegobium paniceum* and/or *Lasioderma serricorne*, which preferably have a minimum size of 2 mm. It is preferred to add the breeding population at the earliest 48 hours and/or at the latest 72 hours after combining the host

population and the support so as to allow the larvae of the beetles to burrow underneath the hard crust and so that preparations for breeding *Spathius exarator* have thus been made. It has been found that, after two to three days, the larvae have burrowed suitably deeply.

It may furthermore be provided that the breeding population is removed from the arrangement after a reproduction time of, in particular 18 - 24 days. After a reproduction time, for example after three weeks, the remaining *Spathius exarator* of the breeding population which are still alive are trapped and removed from the arrangement, in particular from the breeding container. Approximately one week later, the next generation of *Spathius exarator* hatches.

It may additionally be provided that, when using a breeding container, hatched *Spathius exarator* are removed by suction, using a suction tube especially designed for storing the *Spathius exarator*. Thus, it is possible to use a suction tube (exhauster) for trapping and removing the newly-bred *Spathius exarator*, it being feasible that the suction tube is thereafter also used for storage and transport. For example, the hatched *Spathius exarator* can be transferred into a building with timber components or wooden objects infested with woodworm, and the like.

According to the invention, the environment conditions of the arrangement are chosen such that they favour as much as possible the reproduction of the *Spathius exarator*. In this context, a particularly preferred embodiment of the invention provides that, to establish environment conditions which improve the utilization of the host population, a temperature of the arrangement is maintained in a range of from 20-25°C, preferably 23°C, and/or an atmospheric humidity of 50-70%, preferably 60%, is established, and/or a light/dark cycle with light phases longer than the dark phases is used, with the light phases preferably having a duration of 18 hours and the dark phases a duration of 6 hours. Thus, consistently constant laboratory conditions are established for the breeding, a temperature of 23°C, an atmospheric humidity of 60% and so-called long-day conditions, in which 18 hours light and 6 hours dark (L18:D6) are used, having proved to be a very good choice. This allows the rearage to be improved further.

While the breeding population can be obtained for example from correspondingly suitable eclectors (hatcheries), but can also be trapped in the open or ultimately, when the method has already been carried out, can be separated from the result of the method, the fact that the *Spathius exarator* may include pests, in particular mites, which may adversely affect the rearage has proved to be a problem. In an advantageous embodiment of the method it may therefore be provided that the, before being used in the arrangement, are treated against mites, in particular for 11 - 13 hours with a 1% strength beta-naphthol solution in ethanol, which is, preferably on a filter paper, introduced in a quarantine container. It is therefore for example feasible to place the *Spathius exarator* which are used as breeding population into a quarantine breeding container and to treat them against mites for for example 12 hours with 1% beta-naphthol. The beta-nsphthol may be dissolved in ethanol, it being preferred that a specific amount of the solution is added dropwise to a filter paper, which is then dried and introduced into the quarantine breeding container. After a specific treatment time, for example 12 hours, the *Spathius exarator* can be removed from the quarantine breeding container and introduced into the arrangement, which avoids mite infestation and thus a negative effect on the breeding.

The host population can be generated in a breeding batch. As has already been mentioned, bread beetles and/or tobacco beetles, more precisely their larvae, which, owing to a relatively short life cycle, can be bred readily under laboratory conditions, are used. Specifically, it may be provided that a nutrient substrate with 33% bread crumbs, 33% durum wheat semolina, 33% aestivum wheat semolina and 1% glycerol is used in the breeding batch. Such a breeding batch may be stored for example in a glass which is sealed by a piece of fabric which is permeable to air, and an elastic band. After a specified time, for example 6 weeks, the breeding batches can be checked, and, if larvae of a suitable size, as described preferably 2 mm or bigger, are present, they may be taken out.

To this end it may be provided that, for selecting the larvae for the host population, the larvae of the breeding batch are screened with a screen of mesh size 2 mm and the larvae which do not pass through the screen are used as host population. Thus, as soon as larvae of a suitable size can be seen in the breeding batch, the breeding batch is screened through a screen of mesh size 2 mm. Here, exclusively the larger larvae and, if appropriate, some pupae remain in the screen. These larvae are now used as host population for breeding the *Spathius exarator*, while the smaller larvae which have passed through the screen can be reared on. In particular it may be provided that three sequences of screening and rearing processes take place, with in each case one week between the screening processes. The nutrient substrate which remains may also be reused when supplemented with 1% glycerol and when it is stored at -20°C, whereupon, after one week, the nutrient substrate may be reused for fresh breeding batches.

Further advantages and details of the present invention can be seen from the use examples described hereinbelow and with reference to the drawing. The figures show:

Fig. 1 a breeding assembly according to the invention, and

Fig. 2 a baguette section used as support.

Fig. 1 shows, in a schematic diagram, a breeding assembly 1 which can be used for breeding the braconid wasp species *Spathius exarator*. In a breeding container 2, in the present case a fauranium, there is arranged an arrangement 3 which, without the use of the usual host of *Spathius exarator*, which is *Anobium punctatum*, provides an environment in which *Spathius exarator* reproduce.

To this end, the arrangement 3 comprises a support 4, which is a baguette section that has dried for from three to four days into which a host population 5 consisting of larvae of *Stegobium paniceum* and/or *Lasioderma serricorne*, that is to say bread beetles and tobacco beetles, have burrowed. There is furthermore provided a breeding population 6 of *Spathius exarator*.

The support 4, which is formed by the baguette section 7, is shown in greater detail in Fig. 2. It is a section of a traditional baguette whose interior 8 has been freed largely from the soft crumb white and which has then been dried in the air for three to four days. If the baguette section 7 is now populated with larvae of the host population 5, these larvae part into the baguette section 7, which can be seen in Fig. 2 from the holes 9, some of which are shown.

Environment conditions which favour the utilization of the surrogate host, that is the bread beetle and tobacco beetle larvae, by the *Spathius exarator* and thus improve reproduction are maintained by

technical means which are not shown in greater detail, but which are generally known, within the breeding assembly 1, in other words specifically within the breeding container 2. Presently, a temperature of 23°C is maintained at an atmospheric humidity of 60%; moreover, the arrangement 3 is exposed to light for 18 hours and to darkness for 6 hours.

A specific example for breeding *Spathius exarator* using the environment shown in Fig. 1 and Fig. 2 will now be explained in greater detail. Before the actual breeding process starts, the *Spathius exarator* breeding population 6, which may be derived from eclectors or other sources, is first treated against mites. To this end, the *Spathius exarator* are placed into a quarantine breeding container and treated against mites by exposure for 12 hours to beta-naphthol. A 1% beta-naphthol solution in ethanol is prepared, a specific amount, for example 5 ml, of the solution is added dropwise to a paper filter, and this is first dried and placed into the quarantine breeding container. After the 12 hours have elapsed, the *Spathius exarator* are removed from the quarantine breeding container and can be employed for breeding.

As has already been described, it has been found that the surrogate hosts bread beetle and tobacco beetle, which can be bred much more readily under laboratory conditions than *Anobium punctatum*, are accepted by the *Spathius exarator* for reproduction when found in dried baked goods. Thus, an artificial environment for the breeding population 6 is provided in which reproduction, in particular mass breeding, can be realized. To this end, the host population 5 must first be reared.

To this end, a nutrient substrate is used, in the present example a mixture of 33% bread crumbs, 33% durum wheat semolina, 33% aestivum wheat semolina and 1% glycerol. A breeding batch for the host population 5 in a breeding jar contains in the example shown here 100 ml of nutrient substrate and approximately 100 freshly hatched beetles. The breeding jar is sealed with a piece of fabric which is permeable to air, and an elastic band. Every other day, two new breeding batches are started and grown under laboratory conditions which also prevail later within the breeding container 2, thus at 23°C, an atmospheric humidity of 60% and long-day conditions.

After approximately 6 weeks, the breeding batches are checked. If larvae with a size of 2 mm or greater are found at the bottom of the breeding jar, the breeding batch is screened through a screen of mesh size 2 mm. Here, exclusively the larger larvae and, if appropriate, some pupae remain in the screen. These larvae are used as host population 5 for breeding the *Spathius exarator*, while the smaller larvae which have passed through the screen are reared on. After three screening and rearing rounds in each case one week apart, 1% glycerol is added to the remaining nutrient substrate, which is then stored at -20°C, so that, after approximately one week, it can be reused for fresh breeding batches.

The *Spathius exarator* are then bred on the prepared baguette sections 7 where, as has been described, the soft white crumb in the inside 8 is removed, whereupon the hollowed-out baguette sections 7 are dried for three to four days so that ideally a material moisture of 10 to 13% results. The wall thickness is chosen such that it is between 0.8 and 1 cm. If commercially available baguettes for example with a length of 60 cm are divided into three sections and cut open, where it is also possible to discard the upper halves, a dried baguette section 7 can be populated for example with 150 beetle larvae of a

minimum size of 2 mm. After 48 – 72 hours, it can be assumed that the beetle larvae of the host population 5 have burrowed underneath the hard crust so that the baguette sections 7 have reached the state shown in Fig. 2 and can be employed for the breeding.

An exemplary arrangement 3 (breeding batch) in the breeding container 2 may comprise for example four baguette sections 7 and approximately 100 *Spathius exarator*, a mixture of males and females, as the breeding population 6, so that the overall ratio is approximately 100 *Spathius exarator* per 600 larvae; a ratio of 1:6. To supply the *Spathius exarator* with food, cf. Fig. 1, a piece of cotton 10 with a 20% honey-water solution is added, and this piece of cotton is renewed every 48 to 72 hours.

After three weeks, the last *Spathius exarator* of the breeding population 6 which are still alive are then trapped and removed from the breeding container 2, whereupon approximately one week later the new generation of bred *Spathius exarator* hatches, which can then be removed by suction from the breeding container 2 by means of a suction tube. In experiments, approximately 500 *Spathius exarator* were obtained from such a breeding batch as has just been described.

For the sake of completeness, it should be mentioned that presently a faunarium of plastic of approximate dimensions 23 x 15 x 16.5 cm was used. To avoid escape of the animals from the ventilation gaps of the lid 11, a cloth 12, in the present case a commercially available dish cloth, was placed between the lid 11 and the remainder of the breeding container 2.

ÖSSZEÁLLÍTÁS, TENYÉSZTÉSI ELRENDEZÉS ÉS ELJÁRÁS SPATHIUS EXARATOR TENYÉSZTÉSÉRE

Szabadalmi igénypontok

1. Összeállítás (3) *Spathius exarator* tenyésztésére, amely tartalmazza

- a *Spathius exarator* egy tenyészpopulációját (6),
- egy gazdapopulációt (5), és
- egy hordozót (4) a tenyészpopuláció (6) és a gazdapopuláció (5) számára,

ahol

a gazdapopuláció (5) *Stegobium paniceum* és/vagy *Lasioderma serricorne* lárváival rendelkezik és a hordozó (4) kiszárított pékáruból, főként kenyérből áll.

2. Az 1. igénypont szerinti összeállítás, ***azzal jellemezve***, hogy a pékáru legalább három napig van szárítva, főként levegőn.

3. Az 1. vagy 2. igénypont szerinti összeállítás, ***azzal jellemezve***, hogy a tenyészpopuláció (6) tagjainak aránya a gazdapopuláció (5) tagjaihoz képest az 1 : 2 és az 1 : 8 közötti tartományba esik, előnyösen 1 : 6.

4. Az előző igénypontok bármelyike szerinti összeállítás, ***azzal jellemezve***, hogy a lárvák 2 mm-es minimális mérettel rendelkeznek.

5. Az előző igénypontok bármelyike szerinti összeállítás, ***azzal jellemezve***, hogy tartalmaz továbbá egy táplálékforrást a *Spathius exarator* számára, főként egy méz-víz oldattal átitatott vattadarabot (10).

6. Tenyésztési elrendezés (1), amely tartalmaz egy főként műanyagból készült tenyésztőedényt (2), egy az előző igénypontok bármelyike szerinti összeállítással (3).

7. Eljárás *Spathius exarator* tenyésztésére, egy az 1. – 5. igénypontok bármelyike szerinti összeállítás (3) vagy egy a 6. igénypont szerinti tenyésztési elrendezés (1) alkalmazásával.

8. A 7. igénypont szerinti eljárás, ***azzal jellemezve***, hogy a tenyészpopulációt (6) legkorábban 48 órával és/vagy legkésőbb 72 órával a gazdapopuláció (5) és a hordozó (4) összehozása után adjuk hozzá.

9. A 7. vagy 8. igénypont szerinti eljárás, ***azzal jellemezve***, hogy egy szaporodási idő, főként 18 – 24 nap után a tenyészpopulációt (6) eltávolítjuk az összeállításból (3).

10. A 7. – 9. igénypontok bármelyike szerinti eljárás, ***azzal jellemezve***, hogy egy tenyésztőedény (2) alkalmazása esetén a kikelt *Spathius exaratorokat* egy főként a *Spathius exarator* tárolására kialakított szívócsővel szívjuk el.

11. A 7. – 10. igénypontok bármelyike szerinti eljárás, ***azzal jellemezve***, hogy a gazdapopuláció kihasználását javító környezeti feltételek létrehozásához az összeállítás (3) hőmérsékletét 20 és 25 °C közötti tartományban, előnyösen 23 °C-on tartjuk és/vagy 50 és 70 % közötti, előnyösen 60 %-os légnedvességet hozunk létre és/vagy egy olyan fény-sötétség ciklust alkalmazunk, ahol a fényfázisok hosszabbak, mint a sötétségfázisok, és ahol előnyösen a fényfázisok 18 órát, míg a sötétségfázisok 6 órát tartanak.

12. A 7. – 11. igénypontok bármelyike szerinti eljárás, ***azzal jellemezve***, hogy a tenyészpopuláció (6) *Spathius exaratorait* az összeállításban való felhasználás előtt atkák ellen kezeljük, főként 11 – 13 órán keresztül egy 1 %-os béta-naftol oldattal etanolban, amelyet előnyösen egy szűrőpapíron juttatunk egy karantén tartályba.

13. A 7. – 12. igénypontok bármelyike szerinti eljárás, ***azzal jellemezve***, hogy a gazdapopulációt (5) egy tenyészetindító egységben hozunk létre.

14. A 13. igénypont szerinti eljárás, ***azzal jellemezve***, hogy a lárváknak a gazdapopuláció (5) számára való kiválasztásához a tenyészetindító egység lárváit egy 2 mm-es lyukátmérőjű szitával átszűrjük és a szitán át nem jutó lárvákat használjuk fel gazdapopulációként (5).

FIG. 1

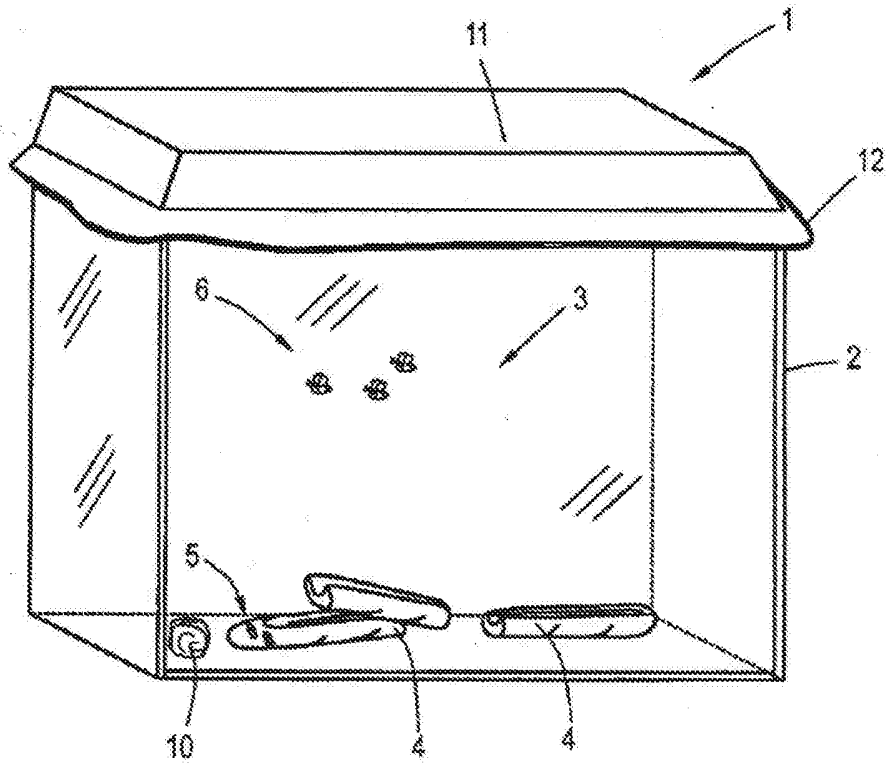

FIG. 2