VÉGÉPHYL – 12ÈME CONFÉRENCE INTERNATIONALE SUR LES RAVAGEURS ET AUXILIAIRES EN AGRICULTURE
26, 27 ET 28 OCTOBRE 2021 - MONTPELLIER SUPAGRO

SORTING OUT PARASITOID COCOONS FROM CLOVER SEED BATCHES: AN INGENIOUS WAY TO UNDERTAKE MASS PARASITOIDS RELEASE IN FIELDS?

B. COUSSY[1], H.B. TOPBJERG [2], F. PAVY [3], J. GERARD [3]

[1]FNAMS - Impasse du Verger, Brain-sur-l'Authion, 49800 Loire-Authion – France – benjamin.coussy@fnams.fr
[2]Department of Agroecology, Faculty for Technical Sciences, Aarhus University, 4200 Slagelse - Denmark
[3]LABOSEM - Impasse du Verger, Brain-sur-l'Authion, 49800 Loire-Authion – France

ABSTRACT
Pests from *Hypera* and *Protapion* genera (Coleoptera) can be harmful to seed clover productions, especially to white clover (*Trifoliumrepens* L.) and red clover (*Trifoliumpratense* L.). Hymenopterans from the*Bathyplectes* genus (Ichneumonidae) parasitise several *Hypera* species and are known to construct recognisable cocoons. Also, these cocoons can be massively found in clover seeds batches after harvest, especially in white clover batches. Trials led in Denmark showed that a suitable sorting protocol allows for mass collection of these cocoons. However, high mortality rate (97%) is observed after sorting and it has not yet been experimentally validated if reintroducing cocoons in fields actually reduces pest populations significantly. In France, sorting has been experimented on red clover seeds batches and seems to work, but it is still not known if *Bathyplectes* can parasitise the main pest on this crop, i.e. *Protapiontrifolii* L. If not, these parasitoids could nevertheless serve as a biocontrol agent against *Hypera* species found on other leguminous crop, i.e. alfalfa (*Medicago sativa* L.).
Keywords: seed sorting, clover, parasitoid, *Bathyplectes*, *Hypera*

RÉSUMÉ
Trier des cocons de parasitoïdes dans des lots de semences de trèfle : une technique ingénieuse pour relâcher massivement des parasitoïdes au champ ?
Plusieurs insectes ravageurs des genres *Hypera* et *Protapion* (Coleoptera) peuvent impacter les productions de semences de trèfles, en particulier de trèfle blanc (*Trifolium repens* L.) et de trèfle violet (*Trifolium pratense* L.). Les hyménoptères du genre *Bathyplectes* (Ichneumonidae) parasitent plusieurs espèces d'*Hypera* et ont la particularité de former des cocons très caractéristiques. Or, ces cocons sont présents en grand nombre dans les lots de semences de trèfle récoltés, en particulier en ce qui concerne le trèfle blanc. Des essais menés au Danemark montrent qu'un protocole de triage adapté permet de récupérer, en masse, ces cocons. Néanmoins, une très forte mortalité (97%) des parasitoïdes est observée après ce triage et il n'a pour l'instant pas été démontré expérimentalement que réintroduire les cocons en parcelles de production permettait de réduire significativement la pression en ravageurs. En France, le triage a été expérimenté sur des lots de trèfle violet et parait fonctionner, mais il reste à préciser si les hyménoptères obtenus sont capables de parasiter le principal ravageur de cette culture, *Protapiontrifolii* L. Dans le cas contraire, les parasitoïdes pourraient potentiellement servir d'agents de lutte contre l'espèce d'*Hypera* ravageur d'une autre légumineuse, la luzerne (*Medicago sativa* L.).
Mots-clés : triage, trèfle, parasitoïde, *Bathyplectes*, *Hypera*

INTRODUCTION

CLOVERS SEED PRODUCTIONS IN FRANCEAND DENMARK

In France in 2019, red clover (*Trifolium pratense* L., 1753) was the first seed clover production in terms of production area with 7 605ha, a hundred times more than white clover (*Trifolium repens* L., 1753) (only 76ha). It is the second fodder seed production behind alfalfa (*Medicago sativa* L., 1753), this one peaking in 2019 at 22 573ha (GNIS, 2020).

In Denmark, white clover is the primary clover seed crop with a production area of 3411ha in 2019. In comparison, red clover was grown on an area of 150ha, being the second most cultivated clover seed crop(Brancheudvalget for Frø, 2019).

CLOVERS SEED PESTS

In Denmark, white clover seed production is affected by the white clover seed weevil (Coleoptera), *Protapion fulvipes* (Geoffroy, 1785), and weevils from the *Hypera* genus: the lesser clover leaf weevil *Hypera nigrirostris* (Fabricius, 1775) and the clover head weevil *Hyperameles* (Fabricius 1792)(Langer and Rohde, 2005; Topbjerg *et al*, 2016).Feeding by these pests can reduce the seed yield by more than 40 or 50%. *P. fulvipes* is usually found in higher numbers than the hyperine weevils, though the damage caused by individual insects is lower than that of*H. Nigrirostris* and *H. meles*(Hansen and Boelt, 2008;Boelt*et al*, 2019).

In Denmark, *P. fulvipes* adults overwinter under dead leaves, in hedges or at the edge of woods (Freeman, 1965 in Hansen and Boelt, 2008). Adults migrate to the host plants in spring, feed until maturity, and then begin to oviposit. Eggs are laid inside the florets, with one egg per floret. The larva develops inside the floret where pupation also takes place (Hansen and Boelt, 2008). Under field conditions, it takes three weeks for the larva to develop and each larva can consume about five seeds in two ovules as they develop (Bovien and Jørgensen, 1934 in Hansen and Boelt, 2008).

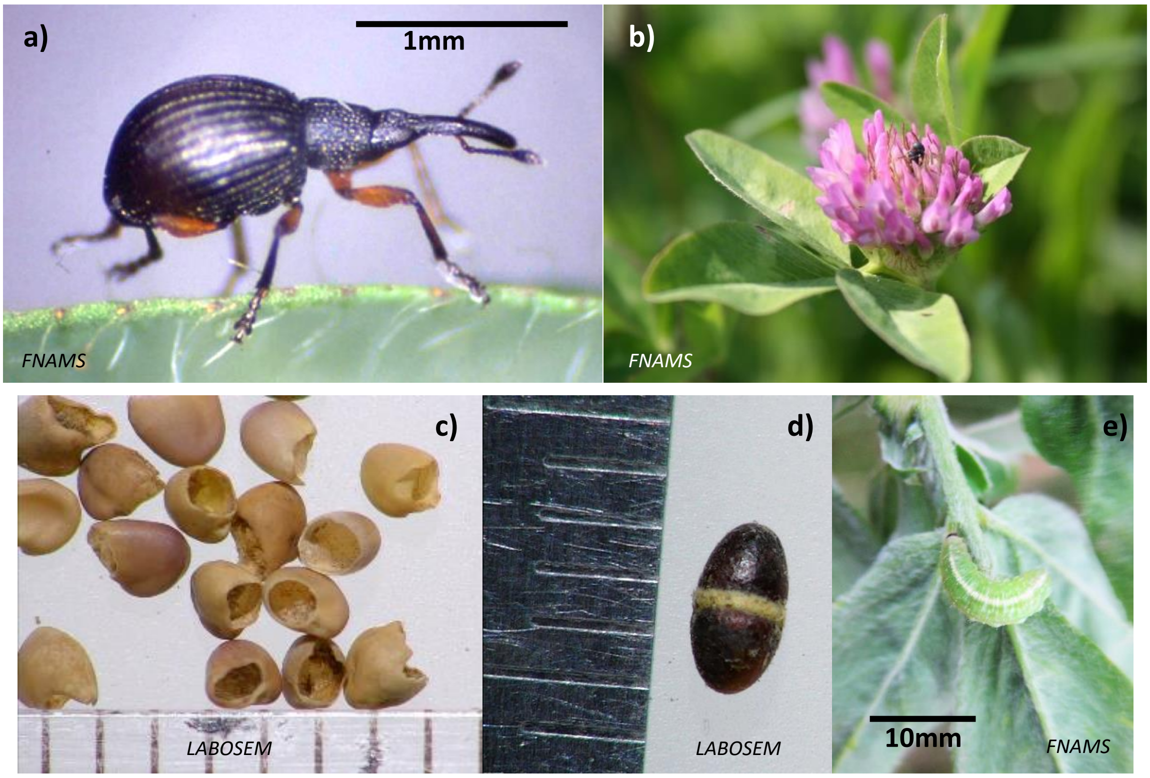
For the hyperine weevils, adults overwinter mainly in clover crops but also, as for *P. fulvipes*, in adjacent areas from where they migrate to the nearest clover crop in the spring. Oviposition occurs in spring and early summer and eggs are laid on or in leaves, petioles, stipules and stems of clover plants. There are 4 larvae instars which develop over a period of three weeks (Hansen and Boelt, 2008). Both larvae and adults can cause damage but larvae inflict the primary damage (Topbjerg *et al*, 2016).After hatching, the larvae migrate to the flowerhead or stem to eat both seeds and green parts of the plant, and in many cases the clover shoot is completely destroyed(Markkula and Tinnila, 1956 in Hansen and Boelt, 2008). Severe infestations cause complete defoliation of clover plants and greatly reduced seed yield(Metcalf *et al*, 1962 in Hansen and Boelt, 2008).*P. fulvipes*, *H. nigrirostris* and *H. meles* have one generation per year (Nyabuga*et al*, 2015; Topbjerg *et al*, 2016).

In France, red clover seed production is highly affected by another insect from the Brentidae family (Coleoptera) but from a different species than in Denmark, *Protapion trifolii* (Linnaeus 1768) (Figure 1a). Fourteen years of experimentations showed that this pest can reduce seed yield by 38% on average (max 70%)(Coussy and Joffre, 2018). Neonicotinoids are an effective way to control this weevil (Kolařík and Rotrekl, 2013; Coussy and Joffre, 2018) but, since their ban in 2018 in France, there is currently no insecticide solution as effective as the neonicotinoids available for red clover fields.

In France, *P. trifolii* overwinters as adults and have one generation per year (FNAMS, 1984). Activity begins in spring (March – April) when adults colonise new fields (Ohlsson, 1968 in Lundin, 2013). In May, ovipositioning is carried out on a specific bud stage, i.e. as the green inflorescence elongates out from the stipule. One egg is laid perflower at a time, though several females can lay on the same flower. During its life span a female can lay 80 to 130 eggs (Agroatlas, 2020).Larvae grow inside the flowerhead, through 3 larval stages with a developmental period of one month. The larva feeds on pollen, ovules or seeds (Figure 1c). A larva can consume on average 8 seeds per flowerhead (FNAMS, 1984) and, usually, several larvae are found in a flowerhead. At the end of feeding, metamorphosis occurs in the flowerhead andnewlyhatched adults emerge during July (FNAMS, 1984). These adults consume leaves but do not lay eggsin the crop. After a few weeks, they leave the red clover field to overwinter in the adjacent environment, and seek towards new fields in the coming spring (Schnell, 1955 in Lundin, 2013).

Other pests that can be found on red clover are insects from the Hyperini tribe, especially *Hyperapostica* (Gyllenhal, 1813), *Brachyperazoilus* (Scopoli, 1763) and, as in Denmark on white clover, *Hypera nigrirostris* (FNAMS, 1984). These insects can be harmful to seed production but are encountered less frequently than *P. trifolii*in the fields.

Figure1: Pictures of (a) *P. trifolii*adult, (b) *P. trifolii* mating on a red clover flowerhead, (c) red clover seeds eaten by *P. trifolii*, (d) *Bathyplectes* sp. cocoon found in red clover, (e) *Hypera* sp. larva on alfalfa
(Photographies (a) d'un adulte de*P. trifolii*, (b) d'un accouplement de *P. trifolii* sur un capitule de trèfle violet, (c) de graines de trèfle violet mangées par *P. trifolii*, (d) d'un cocon de*Bathyplectes*sp. trouvé dans du trèfle violet, (e) d'une larve d'*Hypera*sp.sur luzerne)



PARASITOIDS OF CLOVER WEEVILS

In Sweden, *Spintherus dubius* (Nees, 1834), a small hymenopteran wasp (Pteromalidae), is known to parasitize both *P. fulvipes* and *P. trifolii*(Faraone*et al*, 2017).*S. dubius* has also been observed in the north of France (Vago, 1993) and then should be present in red clover seed fields. After mating, females are active on clover flowers, drumming the florets with the antennae to localize a weevil larva. Once located, an egg is laid inside the larva (medium to late instar), and the parasitoid larva feeds on the host causing its death (Faraone*et al*, 2017).*S. dubius* is likely to have two generations per year and to hibernate as full-grown larvae in the clover heads (Stern *et al*, 2012).

Three species of the genus *Bathyplectes*(Ichneumonidae), *Bathyplectesstenostigma* (Thomson, 1887), *Bathyplectesanurus* (Thomson, 1887) and *Bathyplectescurculionis* (Thomson, 1887) are parasitoids of *Hypera* species, and especially *H.postica*and *H. nigrirostris*(Dysart and Coles, 1971; Shrestha *et al*, 2018). These species oviposit in larval instars of *Hypera* sp., with one parasite larva consuming a single host larva.*B. curculionis* and *B. anurus* ultimately emerge to spin a recognisable white-banded and dark brown cocoon, usually inside the weevils' own cocoon, entering diapause inthe fall. After pupation, it changes into adult and remains in diapause in the cocoon until the next spring (Dysart and Coles, 1971; Roshandel and Ebrahimi, 2003). *B. stenostigma* differs from the others because it exits from the dead host larva to spin its cocoon in the ground litter or inside a curled alfalfa leaf. Furthermore, cocoons from this species have a distinctly different texture from those formed by either *B. curculionis* or *B. anurus* and have no exterior white band (Dysart and Coles, 1971).*Hypera* species and their parasitoids*Bathyplectes* sp. are found on clover crops but also on alfalfa crops (*Medicago sativa* L., 1753) (Figure 1e) (Bartell and Pass, 1980; Takagi *et al*, 2005).

AN INGENOUS WAY TO GET PARASITOIDS

Observations in Denmark has shown that harvested material from white clover seed fields can contain large amounts of parasitoid cocoons. Since 2015, a method to sort these pupae from debris (weed seeds, soil particles and plant debris) has been studied and finally developed. It separates parasitoïd cocoons from other waste materials through different sorting machines (Topbjerg *et al*, 2016). The aim is to collect a large amount of cocoons, that otherwise would have been destroyed with the remaining waste material, and release these cocoons in the clover fields the following spring. This gives a mix between inoculated biological control (bringing cocoons in mass to a location) and conservation biological control (strengthening the numbers of the indigenous population). The method is described and discussed in the following.

This method devised in Denmark has been tested in France on red clover material to assess if the sorting process works and allows for the extraction of the parasitoid cocoons and, if it is the case, what parasitoid species can we observe in red clover fields in France?

MATERIAL AND METHODS

SORTING OUT PARASITOID COCOONS FROM DEBRIS

Hand sorting

In 2018, prior to testing cocoons separation from waste with sorting machines, we sortedparasitoid cocoons by hand from 30 samples of red clover raw material, each sample weighted 25g.Samples were from an experimental plot with 15 non-treated and 15 insecticide-treated samples. It permittedus to evaluate that parasitoid cocoons wereactually present in red clover batches, before using sorting machines.

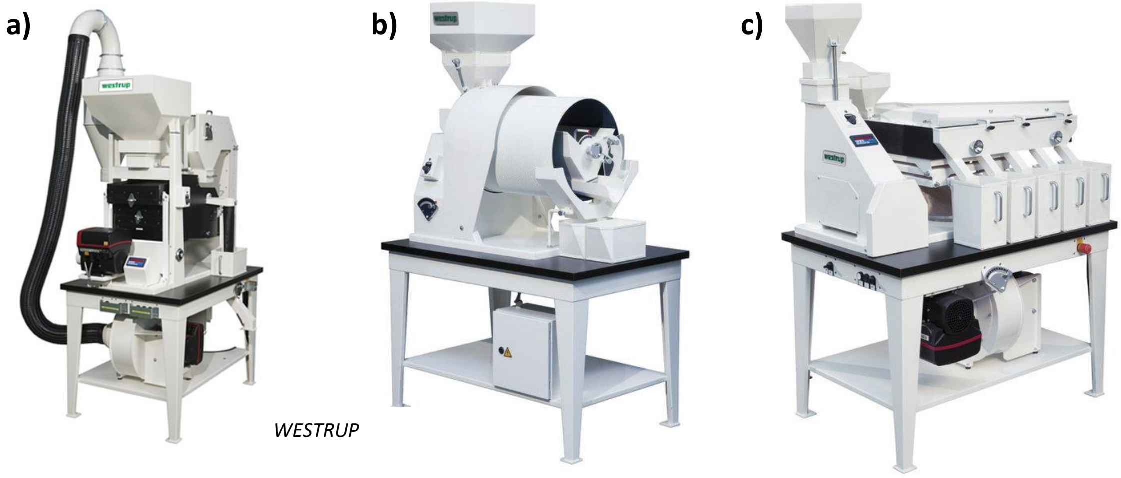
Sorting with machines

In 2019, red clover raw material obtained from another experimental field was used to try out the sorting machines. Three samples of 300 g each were used to evaluate the sorting method which was separatedinto five steps. First step was to discard large and small particles using different sieves and a light suction (Figure 2a). Second step was to remove monocotyledonous weed seeds with an indented cylinder (Figure 2b). Third step was again to discard particles using sieves with a light suction, with sieves having a smaller meshes size than the largest used in the first step and larger than the smallest used in the first step. Fourth step wasa repetition of the third but with a higher suction. The last step was to sort out the cocoons with a gravity separator (Figure 2c).

One important part of the process was the sieves and the fact that they should not be overloaded with material. Adjustments tospeed and shaking depended on the composition of the materialand sorting equipment and was important to achieve a good separation.

We did not find any cocoons in this raw material, so to check that the method was actually discriminant and worked in separating cocoons from debris, we gathered again the raw material and introduced in it 10 cocoons obtained by hand sorting, thus we knew exactly how many cocoons we should find at the end of the process.

Figure2: Pictures of (a) an air-screen cleaner (Westrup LA-LS), (b) an indented cylinder (Westrup LA-T), (c) a gravity separator (Westrup LA-K)
(Photographies (a) d'un nettoyeur air-tamis (Westrup LA-LS), (b) d'un trieur alvéolaire (Westrup LA-T), (c) d'une table densimétrique (Westrup LA-K))

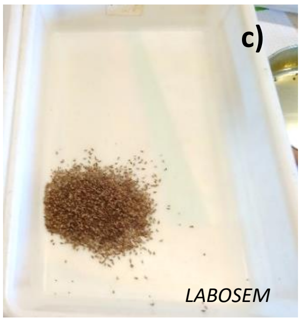


RESULTS

We were able to collect by hand sorting 3,3±2,1 parasitoid cocoons on average for each 25g sample. In 30 samples, only 4 did not contain any cocoons. Surprisingly, mean for the 15 non-treated samples was 1,8±1,4 cocoons and mean for the 15 insecticide-treated samples was 4,9±1,6 cocoons, which is statistically different (Student t-test, df=28, p< 0,0001).

These cocoons did not differ in their aspect and it looks like they are from the same species. Based on the pictures from Dysart and Coles (1971) we can conclude that they are not *B.stenostigma* cocoons, as these do not present an exterior white band. The cocoons we found must therefore either be *B.curculionis* or *B. anurus*, and we assume that they are actually the latter (Figure 1d). Indeed, *B.curculionis*cocoons show a wide, flattened and quite irregular white bands, while those from *B. anurus*show narrow, smooth-edged and slightly raised white bands (Flint *et al*, 1998; Shelton, 2020). This hypothesis should be confirmed with imagos identification but we did not get any imagos from these cocoonsso far.

Figure 3: Pictures of (a) red clover debris after the first step of sorting (different sieves and a light suction), (b) fraction containing cocoons after the first step, (c) fraction containing cocoons after complete sorting (five steps)
(Photographies (a) des déchets de trèfle violet après la première étape du tri (séparateur et ventilation), (b) de la fraction contenant les cocons après la première étape de tri, (c) de la fraction contenant les cocons après le triage complet)



We got raw material from a red clover experimental field, divided into the three samples of 300 g each, and we did not know if cocoons were present in this batch. For each sample, sorting with machines did not provide any cocoons so we did not know if the process was discriminant enough or if there were actually no cocoons. We introduced in one of the samples 10 cocoons obtained by hand sorting, thereby knowing exactly how many cocoons we should find at the end of the process and we sorted it a second time. From the initial 300 g sample (Figure 3, pictures a and b correspond to the whole 300 g sample but split after the first sorting step), we obtained a small batch representing less than 10% of the initial volume (Figure 3c). In this small batch, we found the 10 cocoons introduced at the beginning. We can therefore conclude that the sorting of cocoons in red clover debris with machines works, even if a small amount of particles remain after the complete sorting process.

DISCUSSION

Our survey confirms what has been showed on white clover in Denmark, i.e. there are parasitoid cocoons in red clover debris and that it is possible to extract these cocoons with sorting machines. The method described constitutes five steps, but it might be possible to get the same results with only three steps. These steps would be first to remove monocotyledonous weed seeds with an indented cylinder, then to discard particles using sieves with a significant suction, and finally to get the cocoons with a gravity separator.However, this hypothesis has to be confirmed with further trials.

Concerning *Bathyplectes*species, we assume that we found *B. anurus* cocoons, which is different from the observations on white clover in Denmark where Topbjerg *et al* (2016) identified *B.curculionis*cocoons. But we cannot be sure of our identification because we did not get imagos from these cocoons so far.

We got on average 3,3±2,1 cocoons for 25g, which means that a 100kg red clover raw material could contain 13 333±8 619 cocoons. Topbjerg *et al* (2016) haveestimated in Denmark that an organic white clover seed area of more than 450 ha could theoretically provide more than 26 million parasitoid cocoons. It is a large amount of cocoons but we do not know, in our samples, what proportion is actually viable. It seems that this proportion would be extremely low, around 3% (Topbjerg *et al*, 2016). If we consider the previous extrapolation of 13 333 cocoons in 100 kg, it would represent only 400 viable cocoons. Surveys in progress in Denmark has shown that if the cocoons are obtained directly from the flowerheads in field or from the swath, parasitoid mortality is almost null (Topbjerg *et al*, 2016). So, this mortality must increase in one or several steps between field and sorting. It could be when the swath is drying in field, when the swath is harvested, when the clover seeds are dried to a low water content, when the material is stored just after harvest, when the material is transported to the seed sorting facility, when the debris are sorted out of the raw material or when the cocoons are sorted out of the debris. A process optimization could be achieved if we find which step is detrimental.

In our red clover samples, none of the cocoons we found seemed viable, as we did not observe any imagos emergence in 2018. Since a high mortality rate is observed, it is important to identify a way to separate viable and dead larva within their cocoon. Cocoon weight appears to be a quite good measure and a simple first step to discriminate if the parasitoid inside is still alive or actually dead. Keeping only cocoons heavier than 3,2 mg should allow forat least a viability of 50%in a batch

(Shrestha*et al*, 2018).Shrestha*et al*(2018) also explain that after separation by weight, a multispectral analysis, combining the features of conventional imaging and spectroscopy is able to identify cocoons containing a live larva with an accuracy of 89%.The multispectral imaging system used in the study by Shrestha*et al* (2018) is presented as rapid and non-destructive. Therefore, it could be an efficient way to extract viable cocoons, but it has to be suitable to an industrial sorting.

In France, the main pest of red clover seed production is *P. trifolii*(Coussy and Joffre, 2018). *Bathyplectes* species are not known to parasitize *Protapion* sp., but we actually do not know if parasitism can be possible. The fact that we find *Bathyplectes*cocoons in red clover fields means that the host is present in these fields. It is very likely that the host is a *Hypera* species, but it would be important to evaluate the parasitism rate of *Bathyplectes* on *P. trifolii*.
If we identify that *Bathyplectes* can definitively not parasitize *P. trifolii*, it appears that it is still possible to add value to red clover waste because *Hypera* sp., especially *H. postica*, is a pest of alfalfa seed production and even a major pest in alfalfa fodder production (Roda*et al*, 1996; Boissière and Hacquet, 2011; Coussy, 2019).So, if the cocoons sorting process can be achieved routinely and on an industrial scale, it could be beneficial to alfalfa seed production. Of course, cocoons could also be released in red clover fields against *Hypera* species found on this production, but these species are currently not considered as major pest of *Trifolium pratense*(FNAMS, 1984).
Today, the idea of sorting out the cocoons and using them the following year is still a conceptual idea. Surveys in Denmark are in progress and it is still not clear if releases actually decrease the seed damage by *Hypera* weevils. The increasing in parasitism rates is observed within year of release, but seed yield is not significantly increased nor isthe number of parasitoids available the following year (pers. comm. Topbjerg).

In case of non-parasitism of *Bathyplectes* on*P. trifolii*, it would be interesting to conceive strategies around a known parasitoid of this weevil, as *Spintherus dubius*.The biological information available on the parasitoids in red clover is only fragmentary. We do not know exactly if *S. dubius* hibernates as full-grown larvae in the clover heads (Stern *et al*, 2012), or if females overwinter as adults in drylocations as do other pteromalid species present in red clover (Graham, 1969 in Lundin, 2013). Further investigation is required, nevertheless, specimens may be present in the chaff or elsewhere. Thereby, it seems impossible to proceed as we explain previously with *Bathyplectes* cocoons, i.e. we cannot extract *S. dubius* adults from red clover waste because specimens would probably not survive from the field to the last step of sorting, these specimens being undoubtedly more fragile than cocoons.Diadetchko (1970 in Stern *et al*, 2012) explains that *S. dubius* populations are substantially reduced by seed harvesting, but a large number of hibernating parasitoids remain in the chaff. Therefore, it would theoreticallybe possible to increase the parasitoid abundance by leaving the chaff in piles in the clover fields to permit emergence the following spring.However, due to population increasing of clover root borer *Hylastinus obscurus* (Marsham, 1802) from one year to the next, red clover seed fields in France are, in the majority of cases,conducted only one year, so it is not relevant or possible to leave the chaff for the following year in these cases. To help maintain of parasitoid populations in an area, it is of course important to allow these populations to achieve their biological cycle but also to reduce fragmentation ofhabitats. Kruess and Tscharntke (1994) showed for the specific case of *S. dubius* in red clover that habitat fragmentation affected their populationsize more than thatof theirphytophagous hosts, and the rate of parasitismwas reduced by habitat fragmentation.Maintain habitat connectivity may contributeto the biocontrol of pests, and control strategies of red clover pest should explore this element.

CONCLUSION

Sorting out parasitoid cocoons from debris is an ingenious way to mass collection of biological control agents and to add value to crop waste. It completely depends on the biology of the parasitoid, i.e. if there is a stage where it is in the debris and if we can collect it, but surveys in

Denmark on white clover have shown that this method works and the survey we carried out in France on red clover confirms this. Presently, optimization of the process is necessary for the industry to consider this as an economic gain for the production of clover seeds.
In France, although it might not be beneficial to *P. trifolii* control, this shows that there are still many ways to conceive an agriculture not based on synthetic pesticides, by combining integrated strategies that lead to a durable and biologically stable pest management.

ACKNOWLEDGEMENTS
Authors would like to thank all of the experimenters that allowed us, by their valuable work, to have material to conduct this survey.We also thank our colleagues who patiently sorted out the cocoons of debris by hand.

REFERENCES
Agroatlas, 2020 – Interactive Agricultural Ecological Atlas of Russia and Neighboring Countries. Economic Plants and their Diseases, Pests and Weeds. http://www.agroatlas.ru/en/content/pests/Protapion_trifolii/index.html (accessed June 25, 2020)

Bartell D.P., Pass B.C., 1980 - Morphology, development, and behavior of the immature stages of the parasite *Bathyplectesanurus* (Hymenoptera: Ichneumonidae). *Canadian Entomologist* 112: 481-487.

Boelt B., Topbjerg H.B., Jing S., Gislum R., 2019 - Organic herbage seed production. *Proceedings of the 10th international herbage seed conference*, Corvallis, Oregon, USA - May 12–19, 2019, 21-23.

Boissière L., Hacquet J., 2011 - Ravageurs de la luzerne porte-graine – A chaque région, ses risques. *Bulletin Semences* 218, mars-avril 2011, 30-32.

Brancheudvalget for Frø, 2019 - Beretning 2019. Copenhagen, 33p.

Shelton A., 2020 –Biological Control, A Guide to Natural Enemies in North America. *Bathyplectes anurus* and *B. curculionis* - Hymenoptera: Ichneumonidae. Cornell University. https://biocontrol.entomology.cornell.edu/parasitoids/bathyplectes.php (accessed June 25, 2020)

Coussy B., Joffre A., 2018 - Et maintenant, comment damer l'apion ? *Bulletin Semences* 262, juillet-août 2018, 24-27.

Coussy B., 2019 - Ravageurs de la luzerne porte-graine – Que retenir de dix années de suivis en parcelles de multiplication ? *Bulletin Semences* 268, juillet-août 2019, 25-27.

Dysart R.J., Coles L.W., 1971 – *Bathyplectes stenostigma*, a Parasite of the Alfalfa Weevil in Europe. *Annals of the Entomological Society of America*, 64(6), 1361-1367.

Faraone N., Svensson G.P., Anderbrant O., 2017 - Attraction of the Larval Parasitoid *Spintherus dubius* (Hymenoptera: Pteromalidae) to Feces Volatiles from the Adult *Apion* Weevil Host. J. Insect Behav. (2017) 30:119–129. DOI 10.1007/s10905-017-9605-5.

Flint M.L., Dreistadt S.H., Clark J.K., 1998 - Natural Enemies Handbook: The Illustrated Guide to Biological Pest Control. University of California, Division of Agriculture and Natural Resources, Volume 3386, ISBN-9781879906372. 154p.

FNAMS, 1984 - L'apion du trèfle violet – biologie, importance, mode de lutte. Synthèse des travaux réalisés par le Centre Technique des Semences de la FNAMS, 1979-1984. 50p.

GNIS, 2020 - Groupement National Interprofessionnel des Semences et plants – Etudes et données statistiques. https://www.gnis.fr/etudes-donnees-statistiques-semences/ (accessed June 25, 2020)

Hansen L.M., Boelt B., 2008 - Thresholds of economic damage by clover seedweevil (*Apion fulvipes* Geoff.) and lesser clover leaf weevil (*Hypera nigrirostris* Fab.) on white clover (*Trifolium repens* L.) seed crops. Grass and Forage Science, 63, 433–437.

Kolařík P., Rotrekl J., 2013 - Regulation of the abundance of clover seed weevils, *Apion* spp. (Coleoptera: Curculionidae) in a seed stand of red clover (*Trifolium pratense* L.). Journal of Entomological and Acarological Research, 45(3), e19, 105-109. https://doi.org/10.4081/jear.2013.e19.

Kruess A., Tscharntke T., 1994 - Habitat Fragmentation, Species Loss, and Biological Control. Science, 274, 1581-1584.

Langer V., Rohde B., 2005 - Factors reducing yield of organic white clover seed production in Denmark. Grass and Forage Science, 60, 168–174.

Lundin O., 2013 - Ecology and Management of Crop Pollination and Pest Control - Insights from Red Clover Seed Production. Doctoral Thesis, Swedish University of Agricultural Sciences, Uppsala 2013. 54p.

Nyabuga F.N., Carrasco D., Ranåker L., Andersson M.N., Birgersson G., Larsson M.C., Lundin O., Rundlöf M. ,Svensson G.P., Anderbrant O., LankinenÅ., 2015 - Field Abundance Patterns and Odor-Mediated Host Choice by Clover Seed Weevils, *Apion fulvipes* and *Apion trifolii* (Coleoptera: Apionidae). J. Econ. Entomol. 1–12 (2015); DOI: 10.1093/jee/tou099.

Roda A.L., Landis D.A., Coggins M.L., Spandl E.,Hesterman O.B., 1996 - Forage Grasses Decrease Alfalfa Weevil (Coleoptera: Curculionidae)Damage and Larval Numbers in Alfalfa-Grass Intercrops. *Journal of Economic Entomology*,89, 3, 743-749.

Roshandel S., Ebrahimi E., 2003 -Biology and seasonal parasitism of *Bathyplectes anurus* (Thomson), parasitoid of *Hypera postica* Gill. in Chahar Mahal va Bakhtiari province. *Journal of Entomological Society of IRAN* 22 (2), 1-15.

Shrestha S., Topbjerg H.B., Ytting N.K., Skovgård H., Boelt B., 2018 - Detection of live larvae in cocoons of *Bathyplectes curculionis* (Hymenoptera: Ichneumonidae) using visible/near-infrared multispectral imaging. *Pest Management Science*, 74(9), 2168-2175.

Stern V.M., Adkisson P.L., Beingolea O., Viktorov G.A., 2012 - Cultural *Controls - Theory and Practice of Biological Control*. Elsevier, 2012, ISBN-9780323142441, 810p.

Takagi M., Okumura M., Shoubu M., Shiraishi A., Ueno T., 2005 - Classical biological control of the alfalfa weevil in Japan. Second International Symposium on Biological Control of Arthropods, Davos, Switzerland, September 12-16, 2005: 14-18.

Topbjerg H.B., Ytting N.K., Langer V., Sigsgaard L., Skovgård H., Boelt B., 2016 - An alternative pest management strategy towards weevils in white clover seed production – utilisation of natural enemies. Proceedings of the 16th Nordic herbage seed production seminar - NJF seminar 491. 20 – 22 June 2016, Grimstad, Norway: 74-79.

Vago J.-L., 1993 – *Eurydinota leptomera* Forster et autres chalcidiens ptéromalidés recoltés dans le Pas de Calais (HymenopteraChalcidoideaPteromalidae). *Bulletin de la Société Entomologique du Nord de la France* 267(2) :3-8.