Holistic approach to control *Melolontha* spp. in organic strawberry plantations

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Abstract To achieve an effective reduction of the damage by root feeding grubs of *Melolontha* spp. in organic strawberry plantations, we have tested an approach targeting different stages of the insect’s biological cycle. Adult beetles were caught by using light traps or by manual shaking off trees associated to the monitoring of cockchafer swarm flights supported by forecasts models. Phytosanitary pre-crops and the application of biological control agents were tested against the larvae. The three predictive models utilized to forecast the period of emergence of the cockchafer were suitable to support the deployment of the light traps before the adults’ swarm flights. Traps positioned at 4-m height were more effective in attracting the beetles than those kept at 2-m height. Buckwheat in mixtures with either a mustard or leguminous species used as pre-crops was able to reduce the population of grubs, and considering also its capacity in solubilizing recalcitrant phosphorous sources should enter in a rotation with strawberry or any other crop susceptible to grubs damage. The distribution of two different strains of entomopathogenic fungi resulted in a reduction of the damage to plants due to the cockchafer grubs’ activity, even though the efficacy resulted to be dependent on environmental and agronomic factors, including the kind of formulation used. It is concluded that to assure a sufficient level of control of *Melolontha* spp. in organic strawberry plantations, it is necessary to integrate several methods that are targeting the different biological stages of the insect and are based on different kinds of practices.

Keywords Biological control agents • *Beauveria* spp. • Forecast models • Mass trapping • European cockchafer

Introduction

In recent years, an increase in the damage to nurseries and horticultural crops by root feeding grubs from the forest cockchafer (*Melolontha hippocastani* Fabr.) and the European cockchafer (*Melolontha melolontha* L.) (Coleoptera: Scarabaeidae) has been recorded in Poland and in other European countries (Dolci et al. 2006; Labanowska and Bednarek 2005; Nageleisen et al. 2015). The increase of the populations of these pests in Poland has likely resulted from the concomitant occurrence of different conditions, including the ban of aerial treatments, also to forests and woods, which were used to control the adults of these species, due to the implementation of European Union legal provisions concerned with the sustainable use of pesticides (Directive 2009/128/EC), the lack of chemical products for soil treatments that were previously commonly applied in conventional crops, and the uncultivated afforestation taken over agricultural lands, where grubs have found excellent development conditions (Malinowski 2009). Furthermore, in the past, the beetles’ populations were highly synchronized, leading to years of mass flights.
followed by years when hardly any beetle could be observed. However, currently an overlap of populations is recorded in different countries (Svestka 2010; Wagenhoff et al. 2014). As a result of this situation, the occurrence of extremely severe damage to different horticultural crops, particularly of strawberry and raspberry, but also in blueberry and apple orchards, has become more frequent. The damage to the crops is due to the feeding activity of cockchafer larvae—called white grubs—on the roots of the plants, which negatively affect the uptake of water and nutrients, leading to the wilting and dieback of plants (Zimmermann 2007). Interestingly, strawberry is among the plant species that are highly liked by the grubs to feed on (Huiting et al. 2006).

Several agronomical practices have been proposed to help in controlling the grubs, including soil tillage in association to delayed planting (Woreta 2015) or mulching the soil with fabric-like films as a physical barrier to reduce eggs laying by insects (Tartanus et al. 2017). However, the efficacy of these methods is quite variable and subjected to several factors (e.g., climatic conditions and farming system) which make it difficult to assure a sufficient level of crop protection.

The application of entomophagous organisms is considered an effective method for the biological control of the grubs and has been applied under different climatic and farming systems (Zimmermann 2007). Fungi belonging to the genus Beauveria have been used to develop mycoinsecticides to control pest insects exploiting their ability to specifically infect and kill insects (de Faria and Wraight 2007). Beauveria bassiana (Bals.-Criv.) Vuill. and Beauveria brongniartii (Sacc.) Petch are considered the most suitable species: The former has a wide range of hosts, and the latter is the most prevalent natural antagonist of Melolontha spp. in Europe (Zimmermann 2007). However, the peculiarities of soil management under organic farming, aiming at increasing the soil biological fertility, could increase the competition of autochthonous soil microbial populations toward introduced bioinocula (Hartman et al. 2018), resulting in a reduction of their efficacy.

It is thus emerging that to achieve an effective reduction of damage by grubs of Melolontha spp. in organic strawberry plantations, it is necessary to devise a strategy that should comprise different practices. To this aim, we have tested an approach that targeted the different stages of the insect’s biological cycle: adult beetles by using light traps or by manual shaking off trees associated to the monitoring of cockchafer swarm flights supported by forecasts models, phytosanitary crops preliminary to the planting of the strawberry plants to disturb the growth of grubs in the soil and the application of biological control agents against the larvae. We present here the outcomes of these studies.

Materials and methods

Study site

Field experiments were carried out in an area of the territory of Lubartów district (Lublin voivodeship, South-Eastern Poland), namely, at Brzostówka (51.4365° N, 22.7856° E) (hereafter BZ) and Nowa Wola (51.4177° N, 22.7238° E) (hereafter NW). Fields belonging to members of the association of organic farmers Brzost-Eko dedicated to organic strawberry production were used for the different trials. The site is characterized by organic strawberry and raspberry production (about 50 ha) intercalated by natural woods of deciduous trees, mainly oak. Such condition has traditionally favoured the development of a large population of Melolontha spp. causing severe damage to the crops and the woody trees.

Mass trapping of Melolontha spp. cockchafers

The feasibility of mass trapping was assessed using a light trap devised specifically for this purpose. Each trap consisted of four plastic plates which were placed at a right angle to each other, having the lamp (white light, 12 V, 8 W) in the middle, thereby allowing to gather the attracted beetles in a collector fixed below the plates (Fig. 1a). Ten traps were localized in the vicinity of strawberry plantations and hung on oak trees at two different heights (2 or 4 m, five traps for each option). The traps were emptied six times during the monitoring period (from May 4th till May 30th) and the trapped cockchafers were counted.

Additionally, an attempt to assess the effectiveness of manual shaking of oak trees where the cockchafers fed on was also performed on isolated trees located at the boundary of the plantations which, according to the farmers experience, were commonly infested by cockchafers. The branches up to 5–6 m height of six trees were shaken with a long stick and the falling
cockchafer emergence from soil to support the timely deployment of the mass traps was assessed in 2019 in two locations: BZ and Nowy Dwór, in the surrounding of Skierniewice (51.8637° N, 20.2826° E, Łódź voivodeship, Central Poland). The latter location was established in a field where damages from Melolontha spp. had been reported, so as to verify the suitability of the models under different climatic conditions. The cockchafer emergence (hereinafter the term “emergence” refers to the appearance of adult beetles from their hibernaculae in early spring rather than the eclosion from the pupae, which takes place about 6 months earlier) was surveyed with four soil eclectors per site which had been installed on the ground of fields near the plantations and the woods at the end of April. Each eclector consisted of a gauze net (1 × 1 mm mesh) (Fig. 1b), forming a tent with a square base (each side 4 m) tightly covering the ground area. The eclectors were surveyed, for cockchafer presence, six times starting on 4 May till 30 May, on average every 5 days. The monitoring was conducted by collecting at the same time the daily average air temperature available from a meteorological station located in BZ, in the vicinity of the strawberry plantations, and from the meteorological station present on the experimental station in Nowy Dwór.

The predictive models utilized were previously published by Decoppet (1920), Horber (1955) and Richter (1969). Decoppet (1920) proposed to consider the sum of mean daily air temperatures from 1 March onwards, till the temperature sum exceeds 355 degree days and supposing a minimum temperature threshold of 0 °C to predict the cockchafer emergence from the soil. Horber (1955) proposed to consider the same method but assuming a minimum temperature threshold of 8 °C, with the cockchafer emergence initiating at 256.3 ± 16.3 degree days. The model proposed by Richter (1969) considers 273.5 degree days with 7.7 °C as a threshold for average daily temperature for adult emergence on 15 April. After that day, the required sum of temperature is being reduced by 5.39 °C for each passing day.

Phytosanitary crops for Melolontha spp. grubs

To assess the phytosanitary capacity of a crop (i.e., the detrimental effects of a plant species on the development and weight gain of grubs, Sukovata et al. 2015b) grown before planting strawberries, a trial was carried out comparing three different treatments in 2016: white clover (Trifolium repens) sown in autumn, a mixture composed of pea (Pisum sativum), large-leaved lupine (Lupinus polyphyllus) and buckwheat (Fagopyrum esculentum) or a mixture of buckwheat and white mustard (Sinapis alba), both sown at springtime. Strawberry plants planted the previous spring were considered as control. The size of grubs’ population was assessed by counting the grubs present on 100 m² (four replicates) at the moment of ploughing the soil to prepare it for establishing a new strawberry plantation (August).

Biological control agents against Melolontha spp. grubs

Two entomopathogenic fungal strains of the species Beauveria bassiana and Beauveria brongniartii
were tested as potential biological control agent (BCA) of *Melolontha* spp. soil-dwelling grubs. The strain of *B. brongniartii* (Bbr) was isolated on a selective medium from the soil of a potato field highly infested by *M. melolontha* and was deposited in the Fungal Collection of the Department of Plant Protection and Breeding, Siedlce University of Natural Sciences and Humanities. The strain of *B. bassiana* (Bba) was selected from rhizospheric soil of an apple orchard located in Valle d’Aosta (Italy) by the company CCS Aosta (Aosta, Italy). Both bioinocula were grown in a liquid medium consisting of corn fibres and zeolite (1:10 w-w). In the case of the Bbr strain, a formulation based on barley grains was also used. The concentration of each of the two fungi in the inoculum was about 1·10⁷ CFU g⁻¹.

Two trials were carried out in organic strawberry fields in 2016 and in 2017. Each trial was set with a randomized block design with four replicates (each consisting of 70 or 80 plants for BZ and NW sites, respectively). The BCAs were applied with a dose of 100 kg/ha split in two separate aliquots on the first half of May and second half of June each year (60 kg/ha and 40 kg/ha or 40 kg/ha and 60 kg/ha, for NW and BZ, respectively). Evaluation of efficacy of the treatments was performed by counting the plants showing wilting due to damage to the root system at the end of the period of grubs feeding activity (August 31st).

Statistical analyses

Statistical analysis of data was performed using the R software version 3.5.0 (R Core Team 2019). The Shapiro-Wilk test was used to verify if the data followed a normal distribution, and the Levene’s test was used to verify the homogeneity of variances. The data were thus analysed by ANOVA and means differences tested with Tukey’s test at $p \leq 0.05$ with HSD test function from the “agricolae” package of the R software. In case of not normal distribution, the non-parametric Kruskal-Wallis analysis with Fisher’s least significant difference post hoc test was utilized, introducing the Benjamini-Hochberg correction, with significance set at $p \leq 0.05$, using the `Kruskal` function from the “agricolae” package of the R software.

Results and discussion

Monitoring of cockchafer emergence and mass trapping

According to the meteorological data, the emergence of *Melolontha* spp. population was expected between 20 and 24 April in Central Poland (Nowy Dwór) and between 23 and 26 April in South-Eastern Poland (Fig. 2). In this period, occasional observations of flight activity were reported by the local farmers. However, the first individuals appeared in the ground eclectors 1 week later (4 May) in South-Eastern Poland and 12 days later in Central Poland. A similar delay between the predicted and recorded emergence was reported in South-Western Germany (Wagenhoff et al. 2014), and it could be the result of slight differences due to ground and air temperatures and to the microclimatic conditions of the sites where the eclectors were positioned. The peak of swarming flights was observed about 2 weeks later in both locations in the eclectors (Fig. 3). The results were thus indicating that the three predictive models were useful to forecast the period of emergence of the cockchafer also under Polish conditions. However, the Horber model can be probably considered the most suitable for Poland in defining the time when the swarm would start, still giving some time to the farmers to set the light mass traps before the massive flight of adults occurs.

Following the temperature dynamics calculated with the models, the mass light traps were positioned on the day before the earliest forecasted day of emergence. The first few adults were gathered from the traps on May 4 or 6, in BZ and Nowy Dwór, respectively, from traps positioned at 4-m height (Fig. 3 and Table 1). The trend of the catches by the light traps resulted similar to that of the eclectors. It is worthy to underline, also from a practical point of view, the different efficacy in attracting adults by light traps positioned at the two heights. The light traps hung at 4 m above the ground attracted the largest number of *Melolontha* spp. adults, about twofold than those hung at 2-m height (Fig. 3), particularly when the peak of the swarm flights was observed. However, regardless of their distance from the soil, the traps attracted more males than females in BZ or the opposite in Nowy Dwór (Table 1). Attraction of more males is in agreement with results from other studies (Švestka 2007; Tartanus et al. 2017) and derives from the swarming flights at dusk performed by unpaired males in search of females (Wagenhoff et al. 2014).
Therefore, the outcome in Nowy Dwór could be justified by hypothesizing differences in the populations or in the site landscape which could affect the beetles’ behaviour (Hegedüs et al. 2006; Reinecke et al. 2002). Nevertheless, it should be underlined that employing only ten light traps, we were able to collect in total more than 550 adults in BZ and 704 in Nowy Dwór (with 86 or 612 female cockchafers, respectively). The number of catches per trap was higher than the number of adults that was captured using another kind of light trap, made by a large screen and positioned on the ground (Tartanus et al. 2017), which was also considered too difficult to be utilized as a common practice by farmers. Considering that each female could lay up to 80 eggs, this simple and inexpensive trapping method may, in the medium-term, strongly reduce the grubs’ population in soil and lower the risk of damage to the crop. Furthermore, it should be underlined that monitoring of any pest or the prognosis of its occurrence is an important and critical practice to effectively implement any integrated plant protection strategy (Prasad and Prabhakar 2012), particularly in organic farming and for soil-borne pests, due to the generally lower efficacy of any allowed plant protection product with respect to those authorized in conventional farming.

The identification of the swarming peak allowed also to define the moment for attempting to shake the isolated trees in the vicinity of the plantations to gather adults feeding on them. Using this method, it was possible to collect between 60 and 200 adults per tree at once in few minutes. The method, even though demanding in terms of workload, can be useful particularly for isolated trees or for trees on the external borders of small woods surrounding the plantation. Indeed, other methods to control cockchafers, such as chemical treatments using products based on azadirachtin, present some limitations. Azadirachtin resulted in an inhibition of maturation feeding and a disruption of egg development in female forest cockchafers, but not in immediate mortality (Malinowski et al. 2000; Wagenhoff et al. 2015). Furthermore, for the successful use of this compound, it is crucial to have favourable weather conditions.
during and after the application, necessary to counteract the low persistence of the molecule (Schmutterer 1990) and also to apply it during the peak of female emergence from the soil (Wagenhoff et al. 2015). Thus, these factors, alongside the difficulty in spraying the upper parts of the trees canopy (unpublished observations), hamper the real possibility of a chemical control of the adults’ populations.

Effect of pre-crop on grubs’ abundance in soil

The plant species cultivated on the field before planting the strawberry plants showed to have an influence on the size of the grubs population present in the soil (Table 2). The lowest population of grubs was recorded after growing buckwheat in mixtures with either a mustard or leguminous species. It is noteworthy that the number of grubs found was for both mixtures below the damage threshold for Melolontha spp. (1 grub/ m², Piekarczyk 1993). Clover resulted to have a population of grubs about 10 times higher than the mixtures and potato only twice as much. Strawberry resulted the crop most liked by grubs, with a population about twice and ten times higher than clover or the mixtures with buckwheat, respectively.

Cockchafer white grubs are polyphagous, thus adapted to feed on plants with varying nutritional values. Nevertheless, they seem to have preferences for certain plant species (Schutte 1996; Huiting et al. 2006). Sukovata et al. (2015a) evaluated the impact of different plants, at laboratory scale, on mortality and weight gain of first-instar Melolontha spp. larvae. Grubs fed on clover and lupin roots exhibited a greater weight

| Date       | Number of adult beetles trapped | 4 m aboveground | 2 m aboveground | ground eclector |
|------------|--------------------------------|-----------------|-----------------|-----------------|
|            | ♂♂ ♀♀                           | ♂♂ ♀♀           | ♂♂ ♀♀           | ♂♂ ♀♀           |
| Brzostówka—South-Eastern Poland |                         |                 |                 |                 |
| 4.05       | 10 0                            | 0 0             | 1 0             |
| 8.05       | 13 2                            | 0 0             | 6 2             |
| 17.05      | 150 35                          | 59 35           | 31 37           |
| 21.05      | 96 4                            | 29 6            | 1 0             |
| 25.05      | 28 0                            | 21 1            | 0 2             |
| 30.05      | 1 0                             | 16 3            | 0 1             |
| Total gathered | 298 41                      | 125 45          | 39 42           |
| Nowy Dwór—Central Poland |                          |                 |                 |                 |
| 6.05       | 0 0                             | 0 0             | 10 13           |
| 14.05      | 42 60                           | 10 21           | 54 66           |
| 23.05      | 25 482                          | 3 28            | 13 9            |
| 27.05      | 11 17                           | 1 1             | 0 1             |
| 3.06       | 0 2                             | 0 1             | 0 0             |
| Total gathered | 78 561                       | 14 51           | 77 89           |

Table 2  Effect of pre-crop on the number of Melolontha spp. grubs

| Crop                          | Plot area | Grubs number |
|-------------------------------|-----------|--------------|
|                               | Per 100 m²| Per 2 m²     |
| Strawberry                    | 5000 m²   | 46.00 0.92   |
| Potato                        | 6750 m²   | 5.33 0.11    |
| Clover                        | 4000 m²   | 20.20 0.40   |
| Mixture of lupin, pea and buckwheat | 3150 m²   | 2.41 0.05    |
| Mixture of white mustard and buckwheat | 3150 m²   | 3.01 0.06    |
gain than the control (1-year-old Pinus sylvestris seedlings), and no effect on population mortality was observed. White mustard reduced the mortality of the grubs without additional effect on their weight. On the contrary, buckwheat significantly reduced the grubs’ weight and increased the population mortality. The root content in carbohydrates as well as in polyphenols could account for the different effect of the crops on the grubs’ development and abundance in the soil. Phenolic substances are known to have detrimental effects on insects (Lattanzio et al. 2006). However, their contribution to the disturbance of the grubs’ development has been contradictory (Malinowski 2009; Sukovata et al. 2015a). A different composition of specific secondary metabolites (phenolic compounds and triterpenoids) has been observed in the roots of the tested plants (Malusá et al. in preparation), which could also account for the effects observed in the field.

Under field conditions, the effect of buckwheat thus appeared predominant to that of the leguminous species of the mixtures, being able to reduce the population density of the grubs. Considering the benefits deriving from N-fixing capacity of the leguminous plants and their positive effect on soil fertility (Watson et al. 2002), the association of buckwheat with these species could be considered a suitable option to combine nutrient management and protection from the white grubs in organic strawberry production. The association with Brassica crops could also favour the reduction of pests and diseases due to their content in glucosinolates (Sukovata et al. 2015b). Considering also the ability of buckwheat in solubilizing recalcitrant phosphorous sources (Teboh 2011), it is thus concluded that the introduction of this crop in a rotation with strawberry or any other crop susceptible to grubs damage should become one of the components of a strategy for reduction of Melolontha spp. grubs damage.

Entomopathogenic fungi for the control of cockchafer grubs

The distribution of two different strains of entomopathogenic fungi resulted in a certain reduction of the damage to plants due to the cockchafer grubs’ activity in both sites and seasons considered (Fig. 4). However, the effect was not always statistically significant. The treatment with B. bassiana was the only one showing some plant protection effect in one site (BZ) in 2016, with a reduction of the damage of about 50% in comparison to untreated plants (Fig. 4a). In 2017, both species reduced the damage but to a different extent depending on the formulation used and site (Fig. 4b). However, it should be underlined that the overall average damage in both sites on 2016 was only about 10%, thus making a proper assessment difficult. On the other hand, in 2017 the percentage of damaged plants in untreated plots reached 30% in both sites, and the reduction due to the application of the two BCAs ranged between 15 and 50%. Consequently, the efficacy of the bioinocula ranged between 10 and 49% (Table 3).

Several species and families of agricultural pest insects can be controlled by B. bassiana, while B. brongniartii has been applied mainly against Melolontha spp. (Zimmermann 2007). The assumption made in designing the trials was to verify the feasibility of a treatment based on a more generalist parasite (B. bassiana), which is also more adaptable to different environmental conditions, in comparison to the use of a specific parasite (B. brongniartii), which is, however, less common in the studied sites (Tkaczuk et al. 2014, Tartanus et al. submitted) and thus probably less adapted to their conditions. Moreover, the broader metabolic capacity of the used strain of B. bassiana than B. brongniartii (Canfora et al. 2017) could also suggest its adaptive advantage. The variable efficacy found in both sites for both strains suggests that environmental or agronomical factors can play a key role in shaping the efficacy of the treatment. Possible reasons for reduced

| Treatments | Average efficacy [%] according to Abbott |
|------------|-----------------------------------------|
|            | BZ | NW |
| 2016       |     |    |
| Untreated  | –  | –  |
| B. bassiana water suspension | 52.9 | –15.4 |
| B. brongniartii water suspension | 11.8 | –30.8 |
| 2017       |     |    |
| Untreated  | –  | –  |
| B. bassiana—water suspension | 25.6 | 41.5 |
| B. bassiana—powder | 45.1 | 34.0 |
| B. brongniartii—water suspension | 18.3 | 48.9 |
| B. brongniartii—powder | 26.8 | 9.6 |
| B. brongniartii—grain | 22.0 | 47.9 |
efficacy are related to insufficient fungal density after the application (Keller et al. 2002), application in sandy soils (Keller 2000) as those of the trials, insufficient soil moisture (Walstadt et al. 1970) or soil chemical characteristics such as pH (Weyman-Kaczmarkova and Pedziwilk 2000). Moreover, it should also be underlined that root feeding insects such as cockchafer’s grubs have highly aggregated distributions in the soil, leading to high variability in the belowground infestation levels on the local scale, somehow related to the soil characteristics (Schmidt and Hurling 2014). However, Kessler et al. (2003) found that neither soil water nor organic matter content, under laboratory conditions, was a major factor influencing growth and establishment of B. brongniartii, whereas a negative effect was described for B. bassiana (Studdert and Kaya 1990). It is thus evident that application of BCAs alone is not a sufficient measure to control white grubs, as their efficacy, particularly that of B. brongniartii, has been found to vary depending on the location, the dose or the assessed crop (Dolci et al. 2006; Fătu et al. 2015; Tartanus et al. 2016).

When comparing the two fungal species, no differences were noted in the BZ trial, while B. brongniartii resulted more effective than B. bassiana in the NW trial (Fig. 4). However, it is noteworthy that this occurred when the inoculum was applied after being suspended in water or as a grain formulation, but not when it was applied as a granulated product. The different efficacy observed with the three formulations was previously experienced in pot experiments (unpublished data), and it is in agreement with other field experiments where B. brongniartii applied as water suspension showed higher abundance and persistence in soil (Tartanus et al., submitted). These results underline also the importance of the formulation and application technologies in assuring a sufficient degree of efficacy for microbial-based products (Horaczek and Viernstein 2004; Toegel et al. 2010).

**Conclusions**

To assure a sufficient level of control of Melolontha spp., particularly in organic strawberry plantations, it is necessary to integrate several methods that are targeting the different biological stages of the insect and are based on different kinds of practices. The monitoring of the cockchafers’ swarming period by light traps is an easy and fairly inexpensive method that can also support mass trapping of adults or their collection from woody trees in the vicinity of the plantation. A pre-planting crop such as buckwheat can help to reduce the population of grubs, and also provide additional benefits in solubilizing low-soluble phosphorous. The application of BCAs is a practice which can much reduce the grubs’ damage but should be accompanied by agronomical practices that favour the colonization and persistence of the inoculum. Besides the presented methods, other agronomical practices, such as soil tillage or mulching, can also contribute to reduce the damage, but their effect

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**Fig. 4** Effect of the treatments with entomopathogen fungal strains (Bba, B. bassiana; Bbr, B. brongniartii) on the percentage of strawberry plants damaged on two independent trials (BZ, Brzostówka; NW, Nowa Wola). Means ± SEM, n = 4. Different letters indicate significant differences between treatments within the trial according to the Kruskal-Wallis analysis and Fisher’s least significant difference post hoc test with Benjamini-Hochberg correction, for $p \leq 0.05$. 

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is generally limited or highly dependent on the biological cycle of the pest.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interests.

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