

Susceptibility levels of Hungarian pollen beetle (Coleoptera: Nitidulidae) populations to lambda-cyhalothrin

Magyarországi repcefénybogár (Coleoptera: Nitidulidae) populációk lambda-cihalotrinnal szembeni érzékenysége

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ABSTRACT

The pollen beetle (*Brassicogethes aeneus* Fabricius, 1775) is one of the most harmful pests of winter oilseed rape. Resistant populations have already appeared and spread in Europe due to the misuse of pyrethroid insecticides. Therefore, lambda-cyhalothrin resistance tests were performed on adult beetles collected in four Hungarian habitats according to the coated glass vial assay of the Insecticide Resistance Action Committee (IRAC) in 2018 and 2019. According to the results, all tested individuals were classified into the resistant category, as the mortality at the concentration of 0.075 µg/cm² of the active ingredient, which corresponds to the typical field rate, was only 52–55%. From the cumulated data of the two consecutive years and four sampling sites, the L.D. 50 (0.047 µg/cm²) and the L.D. 90 (0.148 µg/cm²) values of lambda-cyhalothrin were also determined. Total mortality was observed only for the 150% (0.113 µg/cm²) and 200% (0.15 µg/cm²) treatments. Preventing the further spread of pyrethroid-resistant pollen beetle populations and controlling them has become essential in Hungary; therefore, additional monitoring of pollen beetles is critical in developing strategies to address insecticide resistance.

Keywords: *Brassicogethes aeneus*, pyrethroid resistance, monitoring

ÖSSZEFOGLALÁS

A repcefénybogár (*Brassicogethes aeneus* Fabricius, 1775) az őszi káposztarepce egyik legjelentősebb kártevője. A piretroidok túlhasználata miatt, Európa szerte megjelentek és terjednek rezisztens fénybogár populációk. 2018 és 2019 tavaszán, Magyarország négy eltérő adottságú repcetermesztő területéről gyűjtött imágókon végeztünk piretroid (lambda-cihalotrinnal) rezisztencia vizsgálatot, az IRAC módszere alapján. Megállapítottuk, hogy a vizsgált egyedek a rezisztens kategóriába tartoztak, mivel azok tesztben mutatott mortalitása a gyakorlatban alkalmazott dózis (0,075 µg/cm²) esetén csupán 52–55% volt. A vizsgált populációkra a két év és a négy gyűjtési hely összesített adataiból meghatároztuk az LD 50 (0.047 µg/cm²) és az LD 90 (0.148 µg/cm²) értékeket. Teljes mortalitást csak a 150%-os (0,113 µg/cm²) és a 200%-os (0,150 µg/cm²) kezelések során tapasztaltunk. A piretroid-rezisztens repcefénybogár populációk további terjedésének megakadályozása a növényvédelem fontos feladatává vált. Manapság a fénybogarak monitorozása ellengethetetlen a rovarirtó szerekkel szembeni rezisztencia kezelésére szolgáló stratégiák kidolgozásához.

Kulcsszavak: *Brassicogethes aeneus*, repcefénybogár, piretroid rezisztencia, monitoring

INTRODUCTION

The success of winter oilseed rape *Brassica napus* L. (Brassicaceae) cultivation is influenced by several factors, including yield losses caused by pests. Pollen beetles (*Brassicogethes aeneus* Fabricius, 1775) (Coleoptera: Nitidulidae) occurring in early spring are considered harmful to winter oilseed rape. *B. aeneus* is a Holarctic species and a significant pest of oilseed rape and other cruciferous plants in Europe (Sáringer, 1990; Audisio, 1980; 1993; Alford et al., 2003; Ekbom and Borg, 2011). Among other pollen beetle species, *B. aeneus* is dominant, damaging winter oilseed rape in Hungary (Marczali and Keszthelyi, 2003). The females lay their eggs in the buds and flowers of cruciferous plants (Audisio et al., 2000); thus, the adults play a significant role in developing severe damage. In the flower-bud emergence phenological stage, during their maturation feeding, due to access to pollen, they bite sideways into the buds, damaging the genitals, as a result of which the affected buds dry out, fall off, and finally, the flowering shaft becomes bald (Williams and Free, 1978).

Because of their knock-down effect, efficacy and low price, synthetic pyrethroid insecticides are preferred in agriculture. The best-known active ingredients from this group are deltamethrin, cypermethrin, and lambda-cyhalothrin. The latter, used in our experiments, is listed in fifteen different formulations (at the time of drafting the manuscript) approved for controlling pollen beetles in Hungary.

In Hungary, the highest allowed dose for lambda-cyhalothrin is 7.5 g/ha active ingredient. As nerve toxins, pyrethroids (IRAC MoA 3A) act in insects' central nervous system by altering the kinetics of voltage-gated sodium ion channels. As axonic excitotoxins, the toxic effects are based on preventing the closure of the voltage-gated sodium channels in the axonal membranes (Chinn and Narahashi, 1986). When the toxin keeps the channels open, the nerves cannot repolarise, leaving the axonal membrane permanently depolarised, paralysing the target insect (Soderlund et al., 2002).

In addition to their advantages, the selectivity of their mode of action and immediate contact-killing effect can be mentioned. While their disadvantage is that resistance may develop rapidly in the target organisms. The cytochrome P450 enzyme family, which is involved in oxidative metabolism, plays a crucial role in developing resistance to pyrethroids (Zimmer and Nauen, 2011). Unilateral use of pyrethroids can contribute to developing beetle populations with decreased sensitivity. In Germany, in 2005, almost 100% of all insecticides applied to control pests of winter oilseed rape were pyrethroids (Heimbach et al., 2006; Nauen, 2007).

Nowadays, in the traditional rapeseed-growing countries of Europe, the dominance of the pyrethroid-resistant pollen beetle populations can be observed (Ballanger et al., 2003; Wegorek, 2005). The first pyrethroid-resistant population was found in 1999 in the Champagne region of France (Hansen, 2003; 2008). Later, pyrethroid-resistant beetles were reported in Switzerland in 2000 (Derron et al., 2004), in Poland in 2004 (Wegorek, 2005; Wegorek et al., 2006; 2009; Philippou et al., 2011; Zamojska et al. 2013), in Germany in 2002 (Heimbach, 2005; Nauen, 2005).

In 2007, the Insecticide Resistance Action Committee (IRAC) established the Pollen Beetle Working Group to cooperate with European experts and independent researchers to monitor insecticide-resistant pollen beetle populations' development and later spread. In its yearly reports, the working group also called for pyrethroid resistance in Western European countries (Slater et al., 2011).

In Hungary, there is only less data available on the pyrethroid resistance of the pollen beetle; therefore, it was considered essential to get information about the sensitivity of the pollen beetle population to pyrethroids. We aimed to determine the dose of lambda-cyhalothrin ($\mu\text{g}/\text{cm}^2$) needed to achieve total mortality. Furthermore, contrary to the pesticide authorisation documents, the effect of higher doses was also examined.

MATERIALS AND METHODS

In April 2018 and 2019, pyrethroid susceptibility tests were performed in the applied zoological laboratory of the Hungarian University of Agriculture and Life Sciences Georgikon Campus, Institute of Plant Protection. As a test method, a partly modified IRAC insecticide-coated glass vial assay with lambda-cyhalothrin as an active ingredient was used (IRAC, 2009a). The following sampling sites were selected to represent Hungary's main rapeseed growing areas and get a broader picture of each population's pyrethroid sensitivity. Two locations (Győr: 47°38'59.21"N; 17°40'34.76"E; Sorkifalud: 47° 8'2.07" N; 16°44'31.39"E) of the Western Transdanubia region, one from the Southern Transdanubia region (Vése: 46°24'13.48"N; 17°17'57.55"E) and one from Northern Hungary (Szécsény 48° 4'31.27"N; 19°32'4.38"E) were selected (Figure 1).

Pollen beetle adults were collected on 19–25 April 2018 and 12–14 April 2019 before the insecticide sprayings using a sweep net, taking care of the rape field's heterogeneity and collecting at the edges and inside the fields. The samplings were done at the phenological stage 51–61 BBCH of the winter rapeseed. For the experiment's success, a sufficient number of adults (approximately 1000 specimens) were collected at each location. The beetles were stored in plastic bags, taking care not to be exposed to high temperatures. In addition, fresh rapeseed leaves and flowers were placed on dry paper towels in the bags to preserve the beetles' viability.

All the tests were performed at Keszthely on 27 April 2018 and 16 April 2019. Until the tests, the beetles were stored in a refrigerator at 8–10 °C. Then, the beetles were placed into plastic vessels with a densely woven lid at 20–22 °C for approximately one day in the laboratory.

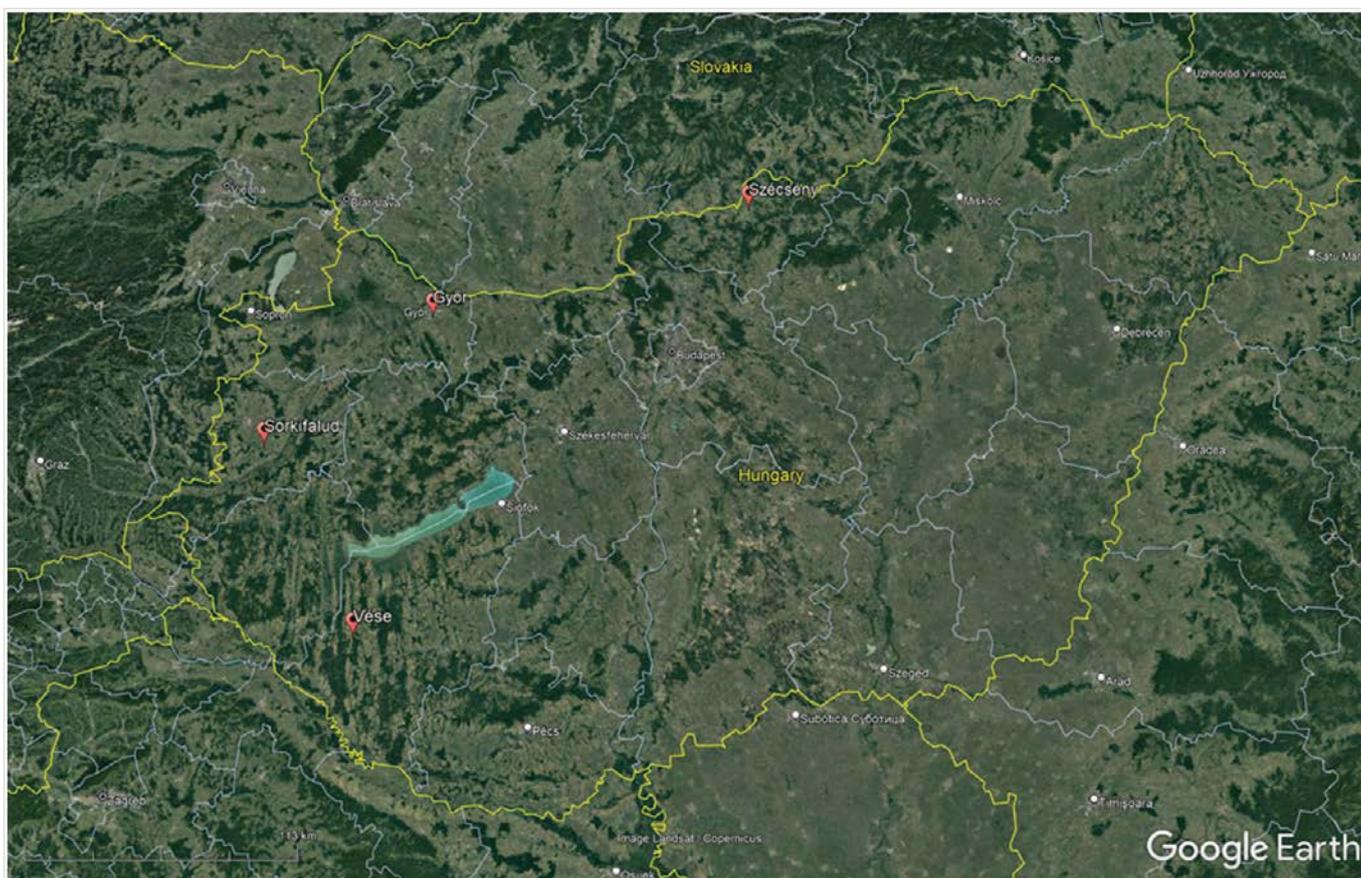


Figure 1. Location of the sampling sites representing the main Hungarian oilseed rape growing areas (Source: Google Earth)

To regain their condition, they were also fed with rapeseed leaves. 20 ml clear glass vials (8.6 cm long and 2.27 cm wide) were prepared for the experiments. Before using the vials, they were washed with soap and water, rinsed with acetone, and dried for four hours. Then, the appropriate concentration of active ingredient was calculated, knowing the surface area of the vials. The bottom plate's surface and the inside of the vial's mantle were considered in the surface calculation ($A = 58.218 \text{ cm}^2$) as the active ingredient only encountered these parts during application. The National Pesticide Analytical Reference Laboratory of Velence provided the pure active substance.

Eight doses in proportion to the typical field rate were used during the laboratory tests. The standard field application rate represented the active ingredient's 100% value and $0.075 \text{ } \mu\text{g}/\text{cm}^2$ concentration. The following doses were used in the tests: 4% ($0.003 \text{ } \mu\text{g}/\text{cm}^2$), 20% ($0.015 \text{ } \mu\text{g}/\text{cm}^2$), 50% ($0.038 \text{ } \mu\text{g}/\text{cm}^2$), 75% ($0.056 \text{ } \mu\text{g}/\text{cm}^2$), 100% ($0.075 \text{ } \mu\text{g}/\text{cm}^2$), 125% ($0.093 \text{ } \mu\text{g}/\text{cm}^2$), 150% ($0.113 \text{ } \mu\text{g}/\text{cm}^2$) and 200% ($0.150 \text{ } \mu\text{g}/\text{cm}^2$), moreover, an untreated control was also used. The original test method was modified by testing three additional doses (125, 150 and 200%) above the 100% concentration to get a more accurate picture of the extent of the potential resistance. All doses were applied in four replicates.

The pure active ingredient was diluted in acetone in a 500 ml standard flask, and the proper amount of the solution was added to the vials using a DragonMed micropipette (100–1000 μl). After that, the acetone was evaporated using a glass spinner in a fume hood for about four hours of rotation. Next, 20 viable adults in good condition were placed in the prepared vials and capped during the test. Care was taken to ensure that all individuals were in contact with the surface covered by the insecticide. After that, the vials were placed in a cupboard (darker place) so that the light could not help the degradation of the active ingredient, and after 24 hours, the mortality assessment started.

During the evaluation, the beetles were placed in the centre of a 15 cm diameter circle drawn from the vial on a white A4 paper sheet. All beetles were examined

in the mortality test. The evaluation was started in solid light, thus stimulating the movement of the beetles. The IRAC's sensitivity classification system distinguishes the following categories:

- Highly susceptible if mortality is 100% for $0,075 \text{ g}/\text{cm}^2$ or 100% for $0,015 \text{ } \mu\text{g}/\text{cm}^2$
- Susceptible if mortality is 100% for $0.075 \text{ } \mu\text{g}/\text{cm}^2$ or <100% for $0.015 \text{ } \mu\text{g}/\text{cm}^2$
- Moderately resistant if mortality is between 90% and 100% at $0.075 \text{ } \mu\text{g}/\text{cm}^2$
- Resistant if mortality is between 50% and 90% at $0.075 \text{ } \mu\text{g}/\text{cm}^2$
- Highly resistant if mortality <50,0% at $0,075 \text{ } \mu\text{g}/\text{cm}^2$.

Analyses were performed using the R statistical programming language (R Core Team, 2019) with the ecotox R package. Because mortality is a variable showing binomial distribution, a generalised linear model (GLM) with a probit link function (Nelder and Wedderburn, 1972) was used instead of the traditional analysis of variance. Probit analysis was used to determine the 50% and 90% lethal doses separately for each year-site-repetition combination.

RESULTS

The results of the lambda-cyhalothrin tests are summarised in Figures 2–4. In the untreated control group ($0 \text{ } \mu\text{g}/\text{cm}^2$), two of the twenty beetles per replication died in two cases, one in ten, and none in the remaining twenty control groups, suggesting the experiment's success. There was no statistical difference in the mortality rates between the treated groups of 4% ($0.003 \text{ } \mu\text{g}/\text{cm}^2$) and the untreated control group. By examining the mortality from the 24-hour post-treatment values, three groups (living, dead, inactive and moribund individuals) were distinguished. The third group is not included in the original method. Its creation was to describe the groups more accurately, characterising the responses 24 hours after the treatments. Moribund individuals who could not move actively were further examined after 24 hours for total mortality. Therefore, only alive and dead individuals were considered for statistical evaluation.

The mortality rate at the 0.075 $\mu\text{g}/\text{cm}^2$ dose was 52–55% for the examined years, which means the adults collected from all sites were resistant. Total mortality was achieved only with the 150% (0.113 $\mu\text{g}/\text{cm}^2$) and 200% treatments (0.150 $\mu\text{g}/\text{cm}^2$) (Figure 2).

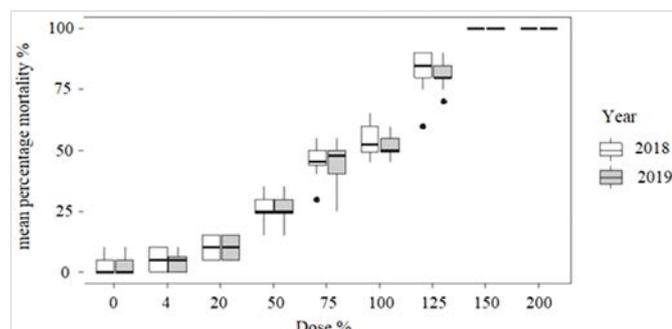


Figure 2. Changes in the mortality rates of pollen beetles on the effect of different doses of lambda-cyhalothrin from 2018 to 2019

Probit analysis was used for each year–site–replication combination to determine the 50% and 90% lethal doses. Since neither the year nor the place significantly affected the mortality, only the concentration, the L.D. 50 and L.D. 90 values were determined from the aggregated data of the four locations and two years (Figures 3–4).

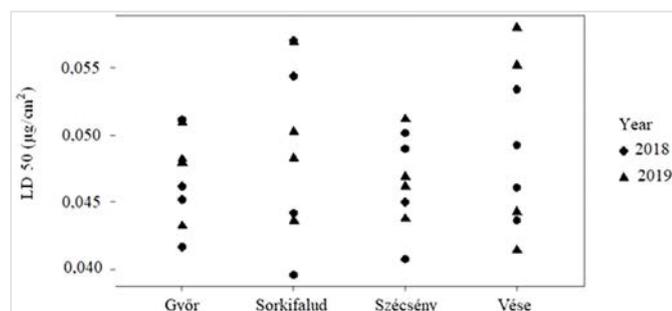


Figure 3. L.D. 50 values calculated from the aggregated data of the four sampling sites in 2018 and 2019

The lowest L.D. 50 value (0.039 $\mu\text{g}/\text{cm}^2$) was measured in Sorkifalud in 2018, while the highest value (0.057 $\mu\text{g}/\text{cm}^2$) was in Vése in 2019. From the aggregated data, the L.D. 50 value was 0.047 $\mu\text{g}/\text{cm}^2$. The lowest L.D. 90 value (0.111 $\mu\text{g}/\text{cm}^2$) was obtained in 2018 in Szécsény, while the highest (0.245 $\mu\text{g}/\text{cm}^2$) was calculated in 2018 in Vése. Based on the summation of the pooled data of the examined years, the L.D. 90 value was 0.148 $\mu\text{g}/\text{cm}^2$.

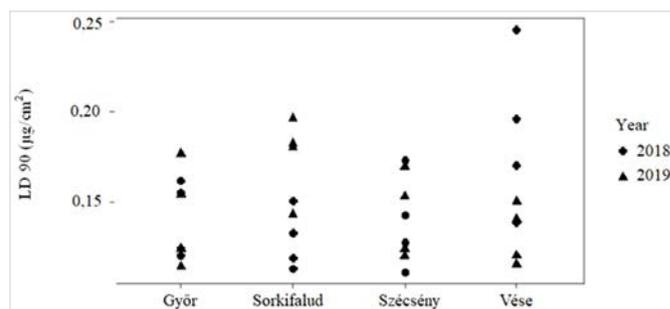


Figure 4. L.D. 90 values calculated from the aggregated data of the four sampling sites in 2018 and 2019

DISCUSSION

In 2007, the IRAC Pollen Beetle Working Group was established to cooperate with European experts and independent researchers to monitor insecticide-resistant pollen beetle populations' development and spread. The very first report of the working group did not refer to any decreased susceptibility of the pest in Hungary. At the same time, highly resistant populations had already developed in France, Belgium, Netherlands, Germany, Switzerland, the United Kingdom and Poland (IRAC, 2008). The following years' report showed that resistant pollen beetle populations remain dominant in France, Germany and Poland. A reduction in pyrethroid-susceptible populations was observed in Switzerland and Austria, with more significant proportions of resistant populations detected. Populations in the United Kingdom remain primarily susceptible. Resistant populations were also identified in Belgium, Czech Republic, Denmark, Estonia, Netherlands, Sweden, Latvia, Lithuania and Luxemburg (IRAC, 2009b). The report in 2010 established that, in general, pyrethroid-resistant populations continued to increase in Central Europe and spread into Eastern Europe, while susceptible populations were still dominant in Hungary (IRAC, 2010). In 2011, resistant populations dominated Western European countries (France, Germany, Denmark, Switzerland) and the Czech Republic, Lithuania and Poland, while significant increases (>10%) in the frequency of resistant populations were observed in Latvia, Lithuania, Finland, Hungary and the U.K. as resistant beetles spread North and North-East. Only Romanian and Ukrainian populations remained fully susceptible to pyrethroids. (IRAC, 2011). It should

be noted that in the case of several countries, a few samples were used (e.g., Hungary and Ukraine). A year later, the report said that only 7% of pollen beetle populations surveyed in Europe can be classified as susceptible to pyrethroids. In all countries surveyed, pyrethroid-resistant pollen beetle populations dominate (>60%). Significant decreases in the percentage of susceptible populations were also observed in the Czech Republic, Hungary and Norway. Hungarian samples (only two) showed susceptible populations (IRAC, 2012). The report published in 2013 noted that only 14% of the European population could be classified as susceptible to pyrethroids. Hungarian populations (only three samples) were classified as moderately resistant (IRAC, 2013). In 2014, only 7% of European populations were considered susceptible, and all Hungarian populations (eight samples) were classified as resistant, with the first appearance of highly resistant populations (IRAC, 2014). In 2015, all Hungarian samples (only three) showed the dominance of resistant populations (IRAC, 2015). A year later, 30% of Hungarian populations were susceptible, 35% were moderately resistant, and 35% were resistant (IRAC, 2016). Our independent preliminary studies performed in 2016 and 2017 showed no decreased susceptibility or resistance (unpublished), while in the IRAC report made in 2017, less than 5% of the Hungarian population was susceptible, about 30% was moderately resistant, 65% was resistant, and 15% was highly resistant. The report suggested that the proportion of resistant populations has stabilised at about 85–90% (IRAC, 2017). In 2018, about 5% of the Hungarian population was susceptible; 20% was moderately resistant, 40% was resistant, and 35% was highly resistant. (IRAC, 2018). In the last IRAC report, half of the Hungarian population was resistant, and the other half was highly resistant (using only two samples) (IRAC, 2019).

It must be considered that the countries and number of samples included in the IRAC surveys varied yearly. The monitoring results would have been much more

reliable if a significantly higher number of samples per country had been processed yearly. Even in our study, we could not represent the susceptibility of all pollen beetle populations in Hungary. The results would have been much more confident with substantially more collection points.

CONCLUSIONS

Over the past fifteen years, monitoring pyrethroid-resistant pollen beetles in Western European countries has been integral to plant protection, urging experts in Hungary to monitor pyrethroid-resistant pollen beetle populations. Our preliminary studies performed in 2016 and 2017 on the exact and near locations showed no decreased susceptibility or resistance (unpublished). A current study found pollen beetle populations with highly reduced sensitivity two years later. This study also demonstrated that lambda-cyhalothrin alone at the highest allowed concentration was no longer suitable for achieving total mortality. To prevent further insecticide resistance development, it is essential to use insecticides with different modes of action, paying particular attention to the possible development of resistance to these active ingredients. As resistant populations dominate and the approvals of resistance-breaking active substances are withdrawn, pest control will become increasingly difficult. Since the study was carried out, the approvals of thiacloprid, chlorpyrifos and indoxacarb have also been withdrawn in the E.U., leaving only type I pyrethroids, type II pyrethroids combined with piperonyl butoxide (PBO) and acetamiprid as resistance-breaking agents.

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