Title

Bumble bee (*Bombus impatiens*) pollination of field crops in the state of California

California Environmental Quality Act
Initial Study and mitigated negative declaration

September, 2006

Prepared for
California Department of Food and Agriculture

Contact:
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Ardea consulting

CEQA Initial Study: Bumble bee (*Bombus impatiens*) pollination of field crops in the state of California.

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1.0 PROJECT DESCRIPTION

1.1 Introduction

The following documents are being presented to the California public per the requirements of the California Environmental Quality Act to inform the public regarding any potential environmental impacts associated with the suggested introduction of *Bombus impatiens* as a pollinator for fruit, nut and vegetable crops in the state of CA. Agriculture is a primary industry and source of income for the state of California. Many seed and fruit crops depend on pollination for adequate production and fruit quality. This project is initiated due to the increasing pressure on the availability of commercially available crop pollinators, which could become a limiting factor for the continued sustainability of California agriculture. Pollination deficits have been reported throughout California due to a lack of honeybees available for rent. Honeybees are currently the only pollinator commercially available in sufficient numbers for the pollination of fruit and vegetable crops. An increase in the rental fee for honeybee hive rentals has been experienced up to the point where prices have become difficult to afford by growers state wide. Observers have questioned the future availability of pollination services for important agricultural crops (Sumner and Boriss, 2006)

As part of these concerns there has been an increasing demand for bumblebees as an alternative in pollination also driven by the following desires of the agricultural community:

- availability of more pollinators (quantity)
- availability of alternative pollinators (quality); and
- price competition between pollinators (economics).

Bumblebees are currently one of very few pollinators commercially available in quantities that could prove to be of help to California agriculture. Bumblebees have previously been evaluated for permitting in covered crops and are currently made available to California greenhouse growers under permit by CDFA. Since the commercially available bumblebee species, *Bombus impatiens*, is not native to California ecologists and biologists have raised potential concerns regarding the release of bumblebees in an outdoor setting. This paper serves as a study to determine the potential impact of the release of a non-native bumblebee, *Bombus impatiens*, into the agro-ecological system of the state of California for crop pollination. In this paper several impacts will be explained and described in detail, mitigations to prevent potential impacts from occurring are introduced as well.

This CEQA document specifically addresses the association between the potential release of bumblebees in California with the requirements described in CEQA. In addition to the CEQA document an ecological risk analysis is given in the APPENDIX, the latter is of broader scope; looking at potential risks that would not necessarily be covered by CEQA definitions.

The setting of this document is the continuing need for pollination and alternative pollinators in California in order for the agricultural businesses in the state to remain sustainable and profitable.

The shortage of pollination capacity and the risks associated with the dependence on a single pollinator; the honeybee (*Apis mellifera)* has been covered in the press over the past few years. In the various parts of this document reference will be made to recent publications discussing the challenges the agricultural industry faces due to the foreseeable lack of pollination capacity and increasing cost of pollination. In addition to the available publications many pleas could be added by individual farmers, their support systems (extension personnel, distributors, etc.) and farming organizations and –groups.

The need and search for alternatives in pollination is not new and the state of California has a history of employing innovative initiatives to overcome pollination shortages and research to implement the available alternatives. To name a few:

- The introduction of the European bee (*Osmia cornuta*) from Spain into California by UC Davis (Torchio and Thorp., 1987) in almond orchards in CA in 1976 and later in 1984
- The research done in CA on an alternative pollinator for alfalfa (*Megachile rotundata*) originating from Manitoba, Canada in 1992 (Peterson et al., 1992)
- The introduction of additional honeybees from outside of the country, source; Australia, since 2002; a use approved by USDA and subsequently by the state of CA (Wehling, 2002).

The perspective of a pressing need for alternatives in pollination and a precedent of providing alternatives based upon research and responsible management will likely be helpful to the public to come to an educated opinion about the proposed use of *Bombus impatiens* in agricultural ecosystems.

1.2 Regulatory Setting

The non native bumblebee *Bombus impatiens* is classified as a plant pest in California and requires a state permit for importation into the state. State permits are issued to California residents or businesses by CDFA; Division of Plant Health and Pest Prevention Services.

1.3 Required Approvals

The California Environmental Quality Act describes the procedure through which any project with a potential environmental impact needs to evaluated. This evaluation needs to take place by a lead agency. This is defined in Article 4 §15051 as;

".. the Lead Agency shall be the public agency with the greatest responsibility for supervising or approving the project as a whole."

In this case approval from CDFA; Division of Plant Health and Pest Prevention Services would be required prior to CDFA approving permits from California residents or businesses.

1.4 Detailed Project Description

Evaluation of the QUAD as a means of introduction of *Bombus impatiens* in outdoor crops is requested under the following pre-requisites:

- Source of *Bombus impatiens* and all constituents; Koppert Biological Systems state of Michigan.
- Timing of introduction; starting week nr. 3 through 22 of the calendar year.
- Pollination duration in the field; Maximum of 5 weeks.
- Pollinator disposal: drowning or freezing after the 5 week pollination period.
- Pollinator packaging: plastic box enclosed by cardboard enclosed by corrugated plastic.
- Introductions exclusively south of 39° latitude in agricultural areas hence the counties:

Yolo	Kings
Sacramento	Tulare
San Joaquin	San Luis Obispo
Solano	Kern
Stanislaus	San Bernardino
Merced	Santa Barbara
Santa Clara	Ventura
Santa Cruz	Orange
San Benito	Riverside
Monterey	San Diego
Fresno	Imperial

Madera

- Use of queen excluders on hives placed inside the QUAD allowing for worker bees to pass freely, but queen bees to remain inside the hive.
- Placement of the QUAD only in the direct vicinity (within 3 feet) of a tall tree or other structure to avoid accidental run-over.
- Establishment of a pollination contract between grower and Koppert noting the prerequisites (mitigations) necessary to be obeyed prior to being eligible to receive QUAD.
- Quality of *Bombus impatiens*; each shipment and production facility inspected by Michigan Department of Agriculture or other state or federal authority for common bee diseases.

2.0 INITIAL STUDY

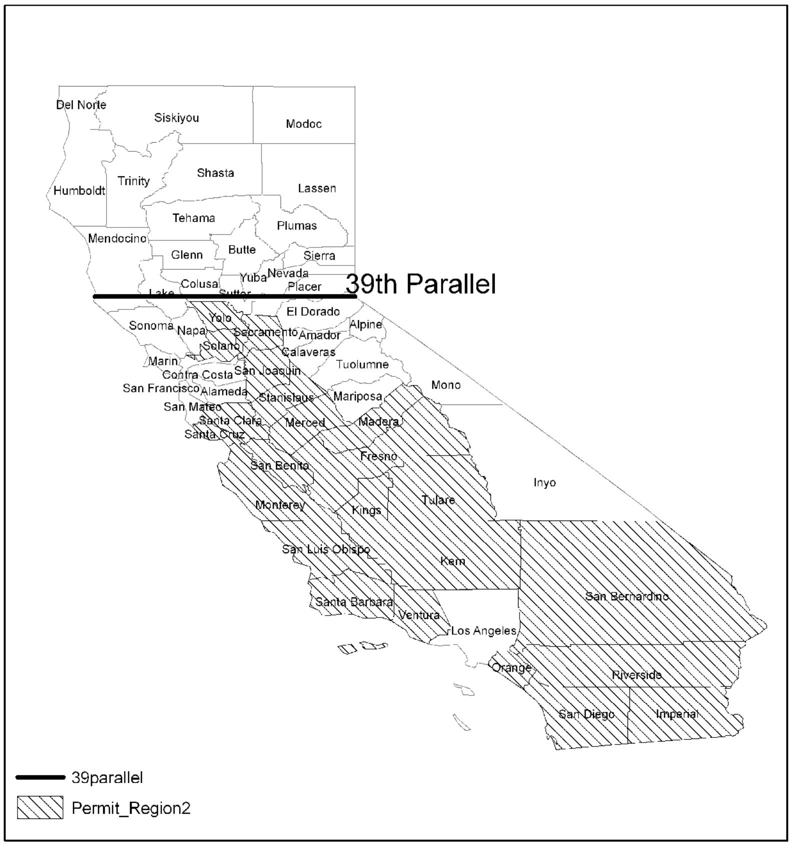
2.1 CEQA Initial Study & Environmental Check List Form

1. Project Title:
Bumble bee (*Bombus impatiens*) pollination of field crops in the state of California

2. Lead Agency Name and Address:
CDFA; Division of Plant Health and Pest Prevention Services

3. Contact Person & Phone Number:
Jim Rains, CDFA. Phone nr.: (916) 651-9371

4. Project Location:
State of California south of 39° latitude in specific counties. See map below. Within those counties introductions will be done by those agricultural corporations or farmers who choose to do so in their agricultural systems such as orchards, vegetable fields and in plastic hoop houses.



5. Other Agencies Whose Approval is Required:
None**.**

2.2 Environmental Factors Potentially Affected

The environmental factor checked below would be potentially affected by the proposed Project, involving at least one impact that is a 'Potentially Significant Impact". The checklist in APPENDIX A has been used to ensure that all areas of concern mentioned in CEQA have been considered.

☐ Aesthetics	☐ Agriculture Resources	☐ Air Quality
☐ Biological Resources	☐ Cultural Resources	☐ Geology/Soils
☐ Hazards & Hazardous Materials	☐ Hydrology/Water Quality	☐ Land Use/Planning
☐ Mineral Resources	☐ Noise	☐ Population/Housing
☐ Public Services	☐ Recreation	☐ Transportation/Traffic
☐ Utilities/Service Systems	☒ Mandatory Findings of Significance	

3.0 EVALUATION OF POTENTIAL SIGNIFICANT IMPACTS

3.1 Definitions

3.1.1. Significant

Paragraph 15382 defines significant impact as:
"Significant effect on the environment" means a substantial, or potentially substantial, adverse change in any of the physical conditions within the area affected by the project, including land, air, water, minerals, flora, fauna, ambient noise, and objects of historic or aesthetic significance.

Significant effect is a relative term that relates the impact of the project (restricted introductions of commercial bumblebee pollinators) to the current situation (commercial availability of pollinators such as honeybees and alfalfa leafcutter bees).

In this analysis the term significant relates to the use of the non-indigenous pollinator *Bombus impatiens* and its impacts as compared to the use of the currently most widely available pollinator *Apis mellifera* and its impacts.

3.1.2. Endangered species

The general concerns related to the release of bumblebees for crop pollination relate to its potential significant effects on endangered native pollinators. Paragraph 15380 defines endangered as follows:

A species of animal or plant shall be presumed to be endangered, rare or threatened, as it is listed in:

(1) Sections 670.2 or 670.5, Title 14, California Code of Regulations; or
(2) Title 50, Code of Federal Regulations Section 17.11 or 17.12 pursuant to the Federal Endangered Species Act as rare, threatened, or endangered.

If a species is not included in any listing identified in subdivision in (1) or (2) it shall nevertheless be considered to be endangered, rare or threatened, if the species can be shown to meet the following criteria:

It can be classified as "Endangered" when its survival and reproduction in the wild are in immediate jeopardy from one or more causes, including loss of habitat, change in habitat, overexploitation, predation, competition, disease, or other factors.

It can be classified as "Rare" when either:

(1) Although not presently threatened with extinction, the species is existing in such small numbers throughout all or a significant portion of its range that it may become endangered if its environment worsens; or
(2) The species is likely to become endangered within the foreseeable future throughout all or a significant portion of its range and may be considered "threatened" as that term is used in the Federal Endangered Species Act.

The definition within CEQA prompts to relate the impact of the project to species listed on the Federal Endangered Species Act: No California pollinating insects are currently on any; rare, threatened, endangered, fully protected or species of special concern list of the California Department of Fish and Game. The U.S. Fish and Wildlife service does not list any bee species in TESS (Threatened and Endangered Species database System).

Individual researchers and NGO's do consider some bee species threatened in the state of CA. The Xerces society for the conservation of pollinators maintains a 'red list' of pollinators it classifies as endangered. Within the state of CA (http://www.xerces.org/Pollinator_Red_List/Table_Bees.htm) it lists:
Bombus franklini
Bombus occidentalis
Halictus harmonius
Protodufourea wasbaueri
Sphecodogastra antiochensis

The first species, *Bombus franklini*, occurs on the CA, OR border about 400 miles from the nearest potential introduction point of the proposed use of bumblebees as commercial crop pollinators. The range of *Bombus occidentalis* does overlap with the proposed use of bumblebees as commercial crop pollinators; Xerces lists *Bombus occidentalis* as potentially endangered with the remark that data is deficient. The latter three species are of a very specific distribution and foraging pattern neither of which is linked to the proposed use of bumblebees for commercial pollination in agro-ecosystems.

In this analysis the term significant impact on endangered species relates to the potential impact of the non-indigenous pollinator *Bombus impatiens* on the native possibly endangered bumblebee *Bombus occidentalis* and related species.

A broader analysis is given in APPENDIX B; An ecological analysis for the use of Bombus impatiens for pollination of field crops in California.

3.2 Areas of impact

The checklist in APPENDIX A is prompted by CEQA and was used to identify any potentially significant environmental impact on the areas mentioned in the definition above. Significance according to the definition (see 3.1.1) was determined for each of the potential concerns Winter et al., (2006) discusses regarding the introduction of a non-native bumblebee; namely

- The transmission of diseases or -parasites by the introduced pollinator.
- Hybridization of the non-native pollinator with native pollinators that are closely related.
- Establishment of the non-native pollinator in the introduced area as an exotic species.
- Competition with native species.

Out of the factors potentially affected one was deemed potentially significant, namely;

	Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact
a) Does the project have the potential to degrade the quality of the environment, substantially reduce the habitat of a fish or wildlife species, cause a fish or wildlife population to drop below self-sustaining levels, threaten to eliminate a plant or animal community, reduce the number or restrict the range of a rare or endangered plant or animal, or eliminate important examples of the major periods of California history or prehistory?	☐	☒	☐	☐

This general concern will be discussed in the following paragraphs as it relates specifically to the potential introduction of the species *Bombus impatiens* into California as compared to the current impact of presently introduced pollinators in agro-ecosystems on any species potentially affected (see 3.1.).

3.3 Transmission of insect diseases or –parasites by the introduced pollinator.

Winter et al. (2006) distinct between the risks associated with exotic pest or disease, which is generally used to refer to pest or disease originating from outside of the US, and pest and disease already established in the US.

Concern has been expressed regarding the observation that imported European bumblebees have carried a parasitic mite into Japan that is genetically different from the native parasitic mite (Goka et al, 2000). Furthermore Winter et al (2006) hypothesizes about a potential link between *Nosema* in commercial hives and the decline in *Bombus franklini* in Oregon. The paper does not indicate whether the declined species had any *Nosema* spores.

The above mentioned observation and hypothesis is the root of a general concern.

3.3.1 Exotic pest and diseases:
Import of bumblebees into the US from any country but Canada has not been allowed for the past number of years and an official prohibition was added in the federal register on oct. 21st 2004, this for the purpose of excluding chances of importing exotic diseases.

Bombus impatiens is a native bee to the US (east of the Rocky Mountains) that has not been reared or in contact with any bumblebees from outside it's native range.

Hence the mitigation to prevent exotic disease transmission into California is as follows:

- Continue to prohibit the importation of bumblebees non-native to North-America and the subsequent exclusive use of bees native to North-America for commercial use in California

Since this condition has been in place in the United States for a number of years there is currently no possibility of bringing in transcontinental pests or diseases.

3.3.2 Pest and disease spread:
Diseases already prevalent in the US are usually not taken into account when a risk analysis is done since these organisms do not pose an added risk; this as stated in the USDA risk analysis for honeybee importation (Wehling, 2002).

However for the purpose of giving a full overview of the concerns it is noted that a process called "pathogen spillover" can occur when a heavily infested 'reservoir' host population spreads its infection to a 'non-host' population (Power and Mitchell, 2004). This process is especially of concern when the host population has a higher incidence of pests or diseases as compared to the non-host population.

When we apply this principle to the potential use of *Bombus impatiens* in outdoor crops in California, we would need to review *Bombus impatiens* from Koppert Biological Systems as the potential host of pests and diseases and native bumblebees as non-hosts.

Infection of bumblebees by pests and parasites influences both the quality as well as the availability (quantity) of hives hence Koppert Biological Systems has implemented a stringent regiment of pest and disease prevention to ensure its capability to continue to sell quality hives to its customers. The detriment of commercial hives would have disastrous effects on the success of rearing facilities, their efficiency and ability to provide sufficient hives for pollination. Koppert's disease prevention regiment is briefly described in APPENDIX C. The result, of this process of disease elimination is independently monitored by the Michigan Department of Agriculture.

Individual certificates are given to those shipments checked by MDA. Examples of this certificate can be reviewed in APPENDIX D.

By ensuring the absence of disease in the hives that Koppert Biological Systems makes available one can ensure that *Bombus impatiens* does not serve as a host of diseases and parasites that could potentially affect natural populations.
Hence the mitigation to prevent disease transmission into California are as follows:

- Commercial use of *Bombus impatiens*, which has proven to posses superior disease resistance properties.
- Each shipment destined for California to be checked by the Michigan Department of Agriculture to ensure absence of pests and diseases and to be certified as ‘clean stock’.

3.3.3 Summary

Bombus impatiens cannot spread exotic diseases from an overseas source into California since it is reared in North-America (Michigan) and the borders have been closed for any bumblebee traffic for a number of years. *Bombus impatiens* could spread other pests or diseases assuming that *Bombus impatiens* referred to in this analysis (*Bombus impatiens* reared in Michigan by Koppert Biological Systems Inc.) have a high incidence of such pests of diseases, higher than the current native species. The use of clean bees can be ensured by the implementation of a rigid pest- and disease management program which, in this case, is independently certified by Michigan state authorities.

3.3.4 CEQA findings of significance

Small hive beetle (*Aethina tumida*) is known to be able to localize, infest and destroy bumblebee colonies (Spiewok and Neumann, 2006). Small hive beetle is a parasite of honeybee colonies and reported to have been transported into California. The transport of this bumble bee pest into California by means of the transport of honey bee hives serves as a potential threat to native bumblebees nesting in the vicinity of apiaries or honey bee hives. Secondly the deformed wing virus, previously reported in honeybees, has been identified in wild *B. pascuorum* in Europe, raising the concern of transmission of viruses between introduced colonies of *Apis melifera* to native bumblebees (Genersch et al. 2006). Both observations indicate the possibility of pest- or disease transmission from honeybees to bumblebees raising the question if there is a significant difference between risks associated with the currently standard practice of introducing honeybees and the alternative of introducing commercial bumblebees for field pollination. Currently about 1 million honeybee hives are being used in CA, indicating that the probability of a potential impact of introduced bumblebee hives on native bee population is likely to be smaller as compared to the impact of the currently introduced honeybee hives. The effect of potential bumble bee introductions, therefore, as it relates to the risk of pest and disease spread is deemed as not significant as compared to the current situation of introduction of honey bees in agro-eco systems.
Specific bumblebee diseases, not transferable from honeybees to bumblebees, potentially introduced via commercial colonies is an additional concern that is mitigated by the use of disease free stock.

3.4 Genetic dilution

3.4.1 Hybridization

For the hybridization of 2 species to occur males and females of these 2 species need be able to locate one another and copulate.

Goulson (2003) identifies 3 strategies that occur in bumblebees to locate a mate:

- Territorial searchers: males wait in a certain territory until a queen enters that territory and they will attempt to mate with her.
- Nest surveillance: males wait at the entrance of the hive until a queen leaves the hive and will attempt to mate with her.
- Scent marking and patrollering: males use pheromones to mark plants and will patrol this area frequently.

Male bumblebees of the species *Bombus impatiens* survey the nest, waiting at the colony entrance until a queen leaves the colony. This behavior restricts the chance of *B. impatiens* males mating with queens from a different species since males will keep watch at the entrances of hives containing queens of their own species.

Pheromones of attraction are species specific even if species are closely related (Goulson, 2003).

The genitals of bumblebees are used for the identification of species. Different species have a different morphology of their reproductive system. The different morphologies between species will restrict chances of interbreeding.

In Kopperts' laboratory experiments to verify the potential for hybridization of closely related species have resulted in no mating or no viable offspring in attempts to mate:

B. impatiens X *B. occidentalis and B. occidentalis X B. impatiens*
B. impatiens X *B. vosnesenskii and B. vosnesenskii X B. impatiens*
B. impatiens X *B. affinis and B. affinis X B. impatiens*
B. impatiens X *B. bimaculatis and B. bimaculatis X B. impatiens*

In a natural setting mating takes place in fall after new queens are leaving their nests, just prior to hibernation. In the proposed commercial application of *B. impatiens* in outdoor situations in California *B. impatiens* males would be venturing from the hive in late spring and early summer; a time in which queens of native bumble bee species will not be available for mating. This timing difference between the availability of native bumblebee queens and *B. impatiens* males will make copulation between species extremely unlikely. Field releases of fully matured colonies prior to wk. 22 will prevent the interaction between males and queens of introduced bees with males and queens of closely related native bees since they would appear at different time frames in nature.

Hence the mitigation to prevent hybridization of *Bombus impatiens* males with native queens or *Bombus impatiens* queens with native males is:

- Use of *Bombus impatiens* pollinators in the time frame that is opposite to the natural cycle of colony development of closely related species.

3.4.2 Summary

Despite the fact that hybridization is highly unlikely; In the proposed usage of *Bombus impatiens* in outdoor agriculture in California it is suggested to only permit the usage of *Bombus impatiens* in a timeframe that would be completely opposite of the natural lifecycle of any native *Bombus spp*. counterparts to prevent interaction of sexuals between introduced and native species.

3.4.3 CEQA findings of significance

Hybridization between *Bombus occidentalis* (see 3.2.2) and *Bombus impatiens* has been attempted but was not successful. It is highly unlikely that these species would interbreed, specifically since they are not of the same subgenus. The risk of mating between *Bombus impatiens* and other native bumblebees in CA is mitigated by restricting placement time frames.

3.5 Establishment

3.5.1 Risk of establishment

Establishment of *Bombus impatiens* in California could have three potential effects;

- a positive effect promoting pollination, fruit set, fruit uniformity and an improved availability of pollinators;
- a neutral effect as in establishment without positive or negative consequences; and
- a negative effect in displacing local native bee species by competition

One cannot associate either outcome with a degree of probability based upon the currently available information.

There is also a potential for the establishment of *Bombus impatiens* in California unless this is deemed undesirable and mitigation is put in place to prevent establishment.

Mitigation to prevent establishment of *Bombus impatiens* West of the Rocky Mountains has apparently been successful with the use of the queen excluder since no establishment of *Bombus impatiens* has been reported in this area despite the use of bumblebees for close to a decade in greenhouses.

Hence the mitigation to prevent establishment of *Bombus impatiens* males is:

- Use of queen excluders on hives placed inside the QUAD allowing for worker bees to pass freely, but queen bees to remain inside the hive.

It should also be noted that the mitigation suggested under ‘hybridization’ will greatly reduce the chance of establishment. We know that diapause is necessary to activate the ‘corporo alata’, an endocrine organism that release hormones that prompt the ovaries to mature (Alford, 1975). In other words without diapause there is no functional reproductive organs in the queen. Diapause induction in the queen is not well understood but diapause is broken by rising temperatures after a winter period. In California these conditions occur at various times depending on the region, but in areas where bumblebees would be likely to be introduced (low lying agriculture ground) these conditions would occur starting wk. 4. If we would assume an escape at the latest date we suggest to introduce bumblebees (wk. 22) and we assume that she was able to mate, feed herself and find a nest that by the time she would come out of hibernation the queen would already have an age of about 7 months leaving a minimal amount of time to build her hive. Furthermore it is known from years of rearing *Bombus impatiens* that the maximum time we can have the queen hibernate and still be able to produce a hive with sexuals is around 26 weeks. Secondly it is interesting to note that the decline of native bumblebee species in the California agro-ecosystem has been primary related to the use of pesticides and the reduction of suitable foraging habitat and hibernation spots through agricultural development. These unfavorable factors for the native bees would just be as unfavorable for any introduced bees. Since bees would exclusively be introduced in the agro-ecosystem to aid in pollination the introduced bees would be subject to a great deal of challenges prior to be able to establish.

3.5.2 Summary

There is no precedent of the establishment of bumblebees in any country in the world where mitigation was put in place. In those places and countries where bumblebees did establish is was either by design or through the authorties not requiring mitigation prior to introduction. In the countries where establishment has taken place no scientific data has linked this established with negative effects in native bumblebees on the population level.

The mitigation proposed to be put in place will greatly reduce the probability of establishment. Furthermore there are several important environmental characteristics of the agro-ecosystem in which bumblebees will be used that will further reduce their chances of survival and hence the chance of establishment. The probability of establishment is deemed to be low.

3.5.3 CEQA findings of significance

The risk of establishment is deemed to be less than significant.

3.6 Competition

The last concern that we will discuss here is that of competition for food sources and habitat between introduced bees and native bees and the potential negative effect that this could have on native bees. For this analysis we make the distinction between two different scenario's:

- competition from introduced hives in the agro-ecosystem; and
- competition from the established non-native bee with native bees

3.6.1 Competition from introduced hives with native bees

The introduced hives would be a growers' purchase done on the basis of trying to prevent a pollination deficit in the field or orchard caused by an overabundance of blooms as compared to what the native bee population can pollinate. This is currently the driving factor behind the introduction of (honey)bee hives. Bumblebees, after placement, forage in the general area in which they are placed. When food is not a limiting factor in the foraging area, competition for food sources does not take place.

3.6.2 Competition from the established non-native bee with native bees

Prior to assessing the probability of competition of an established non-native bee with a native bee we need to note that for this to happen we need to assume that establishment has already happened. Hence the probability of competition would be, by definition, smaller than the probability of establishment unless it is presumed the probability of competition is 1 (it will surely happen).

Here again we should note that competition can only have an effect if we assume that resources are limited in the area where bumblebees are introduced. Since introduced bumblebees, in the case of establishment, would forage and again hibernate in the relative vicinity of their maternal nest.

3.6.3 Summary

The negative effect of competition of food sources presumes that the availability of food sources is limited. In the case of introduction in agro eco-systems with a pollination deficit this would not be the case. In the case of competition by established *Bombus impatiens* the risk of competition is recognized, but per definition smaller than the risk of establishment. Mitigation to prevent competition is the same as the mitigation to prevent establishment.

3.6.4 CEQA findings of significance

The risk of competition is deemed to be less than significant.

4.0 LIST OF MITIGATOIN MEASURES

- Commercial use of *Bombus impatiens*, which has proven to posses superior disease resistance properties.
- Use of *Bombus impatiens* pollinators in the time frame that is opposite to the natural cycle of colony development of closely related species.
- Use of queen excluders on hives placed inside the QUAD allowing for worker bees to pass freely, but queen bees to remain inside the hive.
- Use of the QUAD made up of a sturdy hive box enclosed by a cardboard box enclosed by yet another outdoor box.
- Placement of the QUAD only in the direct vicinity (within 3 feet) of a tall tree or other structure to avoid accidental run-over.
- Establishment of a pollination contract between grower and Koppert noting the prerequisites (mitigations) necessary to be obeyed prior to being eligible to receive QUAD. A proposed pollination contract is included in APPENDIX E.
- Source of *Bombus impatiens* and all constituents; Koppert Biological Systems state of Michigan.
- Quality of *Bombus impatiens*; each shipment and production facility inspected by Michigan Department of Agriculture or other state or federal authority for common bee diseases.
- Timing of introduction; starting week nr. 3 through 22 of the calendar year.
- Pollination duration in the field; Maximum of 5 weeks.
- Pollinator disposal: drowning or freezing after the 5 week pollination period.
- Pollinator packaging: plastic box enclosed by cardboard enclosed by corrugated plastic.
- Introductions south of 39° latitude in agricultural areas hence the counties:

Yolo	Kings
Sacramento	Tulare
San Joaquin	San Luis Obispo
Stanislaus	Kern
Solano	San Bernardino
Merced	Santa Barbara
Santa Clara	Ventura
Santa Cruz	Orange
San Benito	Riverside
Monterey	San Diego
Fresno	Imperial
Madera	

5.0 DETERMINATION (To be completed by lead agency)

On the basis of this initial evaluation:

☐ I find that the proposed project COULD NOT have a significant effect on the environment, and a NEGATIVE DECLARATION will be prepared.

☐ I find that although the proposed Project could have a significant effect on the environment, there will not be a significant effect because appropriate mitigation measures are in place. A MITIGATED NEGATIVE DECLARATION will be prepared.

☐ I find that the proposed project MAY have a significant effect on the environment, and an ENVIRONMENTAL IMPACT REPORT (EIR) is required.

☐ I find that the proposed project MAY have a "potentially significant impact" or "potentially significant unless mitigated" impact on the environment, but at least one effect 1) has been adequately analyzed in an earlier document pursuant to applicable legal standards, and 2) has been addressed by mitigation measures based on the earlier analysis as described on attached sheets. An EIR is required, but it must analyze only the effects that remain to be addressed.

☐ I find that although the proposed project could have a significant effect on the environment, because all potentially significant effects (a) have been analyzed adequately in an earlier EIR or NEGATIVE DECLARATION pursuant to applicable standards, and (b) have been avoided or mitigated pursuant to that earlier EIR or NEGATIVE DECLARATION, including revisions or mitigation measures that are imposed upon the proposed project, nothing further is required.

Signature	Date

Printed name	For Lead Agency

6.0 PERSONS AND AGENCIES CONTACTED

For the preparation of this document the following persons have been interviewed:

Robbin W. Thorp, Ph.D.
Professor Emeritus
UC Davis Department of entomology

Michael Parella, Ph. D.
Associate Dean of Agricultural and Environmental Sciences and Professor of Entomology
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7.0 LIST OF PREPARERS

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8.0 LITERATURE CITED

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APPENDIX A: Checklist of environmental impacts

1 Aesthetics

	Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact
Would the Project:				
a) Have a substantial adverse effect on a scenic vista?	☐	☐	☐	☒
b) Substantially damage scenic resources, including, but not limited to, trees, rock outcroppings, and historic buildings within a state scenic highway?	☐	☐	☐	☒
c) Substantially degrade the existing visual character or quality of the site and its surroundings?	☐	☐	☐	☒
d) Create a new source of substantial light or glare which would adversely affect day or nighttime views in the area?	☐	☐	☐	☒

Discussion

The placement of QUAD hive boxes in agricultural areas will not have a significant impact on any aesthetics.

2 Agriculture Resources

	Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact
Would the Project:				
a) Convert Prime Farmland, Unique Farmland, or Farmland of Statewide Importance (Farmland), as shown on the maps prepared pursuant to the Farmland Mapping and Monitoring Program of the California Resources Agency, to non-agricultural use?	☐	☐	☐	☒
b) Conflict with existing zoning for agricultural use, or a Williamson Act contract?	☐	☐	☐	☒
c) Involve other changes in the existing environment, which due to their location or nature, could result in conversion of Farmland, to non-agricultural use?	☐	☐	☐	☒

Discussion

Utilization of crop pollinators does not influence the conversion of agricultural resources. Pollination supports the sustainability of agricultural resources.

3 Air Quality

		Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact
	Would the Project:				
a)	Conflict with or obstruct implementation of the applicable air quality plan?	☐	☐	☐	☒
b)	Violate any air quality standard or contribute substantially to an existing or projected air quality violation?	☐	☐	☐	☒
c)	Result in a cumulatively considerable net increase of any criteria pollutant for which the project region is non-attainment under an applicable federal and state ambient air quality standard (including releasing emissions which exceed quantitative thresholds for ozone precursors)?	☐	☐	☐	☒
d)	Expose sensitive receptors to substantial pollutant concentrations?	☐	☐	☐	☒
e)	Create objectionable odors affecting a substantial number of people?	☐	☐	☐	☒

Discussion

4 Biological Resources

		Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact
	Would the Project:				
a)	Have a substantial adverse effect, either directly or through habitat modifications, on any species identified as a candidate, sensitive, or special status species in local or regional plans, policies, or regulations, or by the California Department of Fish and Game or U.S. Fish and Wildlife Service?	☐	☐	☐	☒
b)	Have a substantial adverse effect on any riparian habitat or other sensitive natural community identified in local or regional plans, policies, regulations or by the California Department of Fish and Game or U.S. Fish and Wildlife Service?	☐	☐	☐	☒
c)	Have a substantial adverse effect on federally protected wetlands as defined by Section 404 of the Clean Water Act (including, but not limited to, marsh, vernal pool, coastal, etc.) through direct removal, filling, hydrological interruption, or other means?	☐	☐	☐	☒
d)	Interfere substantially with the movement of any native resident or migratory fish or wildlife species or with established native resident or migratory wildlife corridors, or impede the use of native wildlife nursery sites?	☐	☐	☐	☒
e)	Conflict with any local policies or ordinances protecting biological resources, such as a tree preservation policy or ordinance?	☐	☐	☐	☒
f)	Conflict with the provisions of an adopted Habitat Conservation Plan, Natural Community Conservation Plan, or other approved local, regional, or state habitat conservation plan?	☐	☐	☐	☒

Discussion

The biological and ecological concerns associated with the release of a non-native bumblebee are discussed in chapter 3. The bumblebee's potential negative impact as described in chapter 3 would be on native, closely related, bumblebee species. No California insects are currently on any; rare, threatened, endangered, fully protected or species of special concern list of the California Department of Fish and Game. The U.S. Fish and Wildlife service does not list any bee species in TESS (Threatened and Endangered Species database System). However individual researchers and NGO's do consider some bumblebee species threatened in the state of CA.

5 Cultural Resources

	Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact
Would the Project:				
a) Cause a substantial adverse change in the significance of a historical resource as defined in §15064.5?	☐	☐	☐	☒
b) Cause a substantial adverse change in the significance of an archaeological resource pursuant to §15064.5?	☐	☐	☐	☒
c) Directly or indirectly destroy a unique paleontological resource or site or unique geologic feature?	☐	☐	☐	☒
d) Disturb any human remains, including those interred outside of formal cemeteries?	☐	☐	☐	☒

Discussion

6 Geology and Soils

	Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact

Would the Project:

a) Expose people or structures to potential substantial adverse effects, including the risk of loss, injury, or death involving:	☐	☐	☐	☒
i) Rupture of a known earthquake fault, as delineated on the most recent Alquist-Priolo Earthquake Fault Zoning map issued by the State Geologist for the area or based on other substantial evidence of a known fault? Refer to Division of Mines and Geology Special Publication 42.	☐	☐	☐	☒
ii) Strong seismic-related ground shaking?	☐	☐	☐	☒
iii) Seismic-related ground failure, including liquefaction?	☐	☐	☐	☒
iv) Landslides?	☐	☐	☐	☒
b) Result in substantial soil erosion or the loss of topsoil?	☐	☐	☐	☒
c) Be located on a geologic unit or soil that is unstable, or that would become unstable as a result of the project, and potentially result in on- or off-site landslide, lateral spreading, subsidence, liquefaction or collapse?	☐	☐	☐	☒
d) Be located on expansive soil, as defined in Table 18-1-B of the Uniform Building Code (1994), creating substantial risks to life or property?	☐	☐	☐	☒
e) Have soils incapable of adequately supporting the use of septic tanks or alternative wastewater disposal systems where sewers are not available for the disposal of wastewater?	☐	☐	☐	☒

Discussion

7 Hazards and Hazardous Materials

	Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact
Would the Project:				
a) Create a significant hazard to the public or the environment through the routine transport, use, or disposal of hazardous materials?	☐	☐	☐	☒
b) Create a significant hazard to the public or the environment through reasonably foreseeable upset and accident conditions involving the release of hazardous materials into the environment?	☐	☐	☐	☒
c) Emit hazardous emissions or handle hazardous or acutely hazardous materials, substances, or waste within one-quarter mile of an existing or proposed school?	☐	☐	☐	☒
d) Be located on a site which is included on a list of hazardous materials sites compiled pursuant to Government Code Section 65962.5 and, as a result, would it create a significant hazard to the public or the environment?	☐	☐	☐	☒
e) For a project located within an airport land use plan or, where such a plan has not been adopted, within two miles of a public airport or public use airport, would the project result in a safety hazard for people residing or working in the project area?	☐	☐	☐	☒
f) For a project within the vicinity of a private airstrip, would the project result in a safety hazard for people residing or working in the project area?	☐	☐	☐	☒
g) Impair implementation of or physically interfere with an adopted emergency response plan or emergency evacuation plan?	☐	☐	☐	☒
h) Expose people or structures to a significant risk of loss, injury or death involving wildland fires, including where wildlands are adjacent to urbanized areas or where residences are intermixed with wildlands?	☐	☐	☐	☒

Discussion

8 Hydrology and Water Quality

	Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact

Would the Project:

	Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact
a) Violate any water quality standards or waste discharge requirements?	☐	☐	☐	☒
b) Substantially deplete groundwater supplies or interfere substantially with groundwater recharge such that there would be a net deficit in aquifer volume or a lowering of the local groundwater table (e.g., the production rate of pre-existing nearby wells would drop to a level which would not support existing land uses or planned uses for which permits have been granted)?	☐	☐	☐	☒
c) Substantially alter the existing drainage pattern of the site or area, including through the alteration of the course of a stream or river, in a manner which would result in substantial erosion or siltation on- or off-site?	☐	☐	☐	☒
d) Substantially alter the existing drainage pattern of the site or area, including through the alteration of the course of a stream or river, or substantially increase the rate or amount of surface runoff in a manner which would result in flooding on-or off-site?	☐	☐	☐	☒
e) Create or contribute runoff water which would exceed the capacity of existing or planned stormwater drainage systems or provide substantial additional sources of polluted runoff?	☐	☐	☐	☒
f) Otherwise substantially degrade water quality?	☐	☐	☐	☒
g) Place housing within100-year flood hazard area as mapped on a federal Flood Hazard Boundary or Flood Insurance Rate Map or other flood hazard delineation map?	☐	☐	☐	☒
h) Place within a 100-year flood hazard area structures which would impede or redirect flood flows?	☐	☐	☐	☒
i) Expose people or structures to a significant risk of loss, injury or death involving flooding, including flooding as a result of the failure of a levee or dam?	☐	☐	☐	☒
j) Inundation by seiche, tsunami, or mudflow?	☐	☐	☐	☒

9 Land Use Planning

	Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact

Would the Project:

a) Physically divide an established community?	☐	☐	☐	☒
b) Conflict with any applicable land use plan, policy, or regulation of an agency with jurisdiction over the project (including, but not limited to the general plan, specific plan, local coastal program, or zoning ordinance) adopted for the purpose of avoiding or mitigating an environmental effect?	☐	☐	☐	☒
c) Conflict with any applicable habitat conservation plan or natural community conservation plan?	☐	☐	☐	☒

Discussion

10 Mineral Resources

	Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact

Would the Project:

a) Result in the loss of availability of a known mineral resource that would be of value to the region and the residents of the state?	☐	☐	☐	☒
b) Result in the loss of availability of a locally-important mineral resource recovery site delineated on a local general plan, specific plan other land use plan?	☐	☐	☐	☒

Discussion

11 Noise

	Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact

Would the Project result in:

a) Exposure of persons to or generation of noise levels in excess of standards established in the local general plan or noise ordinance, or applicable standards of other agencies?	☐	☐	☐	☒
b) Exposure of persons to or generation of excessive groundborne vibration or groundborne noise levels?	☐	☐	☐	☒
c) A substantial permanent increase in ambient noise levels in the project vicinity above levels existing without the project?	☐	☐	☐	☒
d) A substantial temporary or periodic increase in ambient noise levels in the project vicinity above levels existing without the project?	☐	☐	☐	☒
e) For a project located within an airport land use plan or, where such a plan has not been adopted, within two miles of a public airport or public use airport, would the project expose people residing or working in the project area to excessive noise levels?	☐	☐	☐	☒
f) For a project within the vicinity of a private airstrip, would the project expose people residing or working in the project area to excessive noise levels?	☐	☐	☐	☒

Discussion

12 Population and Housing

	Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact

Would the Project:

a) Induce substantial population growth in an area, either directly (for example, by proposing new homes and businesses) or indirectly (for example, through extension of roads or other infrastructure)?	☐	☐	☐	☒
b) Displace substantial numbers of existing housing units, necessitating the construction of replacement housing elsewhere?	☐	☐	☐	☒
c) Displace substantial numbers of people, necessitating the construction of replacement housing elsewhere?	☐	☐	☐	☒

Discussion

13 Public Services

	Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact
a) Would the project result in substantial adverse physical impacts associated with the provision of new or physically altered governmental facilities, need for new or physically altered governmental facilities, the construction of which could cause significant environmental impacts, in order to maintain acceptable service ratios, response times or other performance objectives for any of the public services:	☐	☐	☐	☒
Fire protection?	☐	☐	☐	☒
Police protection?	☐	☐	☐	☒
Schools?	☐	☐	☐	☒
Parks?	☐	☐	☐	☒
Other public facilities?	☐	☐	☐	☒

Discussion

14 Recreation

		Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact
a)	Would the project increase the use of existing neighborhood and regional parks or other recreational facilities such that substantial physical deterioration of the facility would occur or be accelerated?	☐	☐	☐	☒
b)	Does the project include recreational facilities or require the construction or expansion of recreational facilities which might have an adverse effect on the environment?	☐	☐	☐	☒

Discussion

15 Transportation/Traffic

	Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact

Would the Project:

a) Cause an increase in traffic which is substantial in relation to the existing traffic load and capacity of the street system (i.e., result in a substantial increase in either the number of vehicle trips, the volume to capacity ratio on roads, or congestion at intersections)?	☐	☐	☐	☒
b) Exceed, either individually or cumulatively, a level of service standard established by the county congestion management agency for designated roads or highways?	☐	☐	☐	☒
c) Result in a change in air traffic patterns, including either an increase in traffic levels or a change in location that results in substantial safety risks?	☐	☐	☐	☒
d) Substantially increase hazards due to a design feature (e.g., sharp curves or dangerous intersections) or incompatible uses (e.g., farm equipment)?	☐	☐	☐	☒
e) Result in inadequate emergency access?	☐	☐	☐	☒
f) Result in inadequate parking capacity?	☐	☐	☐	☒
g) Conflict with adopted policies, plans, or programs supporting alternative transportation (e.g., bus turnouts, bicycle racks)?	☐	☐	☐	☒

Discussion

16 Utilities and Service Systems

	Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact

Would the Project:

a)	Exceed wastewater treatment requirements of the applicable Regional Water Quality Control Board?	☐	☐	☐	☒
b)	Require or result in the construction of new water or wastewater treatment facilities or expansion of existing facilities, the construction of which could cause significant environmental effects?	☐	☐	☐	☒
c)	Require or result in the construction of new storm water drainage facilities or expansion of existing facilities, the construction of which could cause significant environmental effects?	☐	☐	☐	☒
d)	Have sufficient water supplies available to serve the project from existing entitlements and resources, or are new or expanded entitlements needed?	☐	☐	☐	☒
e)	Result in a determination by the wastewater treatment provider which serves or may serve the project that it has adequate capacity to serve the project's projected demand in addition to the provider's existing commitments?	☐	☐	☐	☒
f)	Be served by a landfill with sufficient permitted capacity to accommodate the project's solid waste disposal needs?	☐	☐	☐	☒
g)	Comply with federal, state, and local statutes and regulations related to solid waste?	☐	☐	☐	☒

Discussion

17Mandatory Findings of Significance

	Potentially Significant Impact	Potentially Significant Unless Mitigation Incorporated	Less Than Significant Impact	No Impact
a) Does the project have the potential to degrade the quality of the environment, substantially reduce the habitat of a fish or wildlife species, cause a fish or wildlife population to drop below self-sustaining levels, threaten to eliminate a plant or animal community, reduce the number or restrict the range of a rare or endangered plant or animal, or eliminate important examples of the major periods of California history or prehistory?	☐	☒	☐	☐
b) Does the project have impacts that are individually limited, but cumulatively considerable? ("Cumulatively considerable" means that the incremental effects of a project are considerable when viewed in connection with the effects of past projects, the effects of other current projects, and the effects of probable future projects)?	☐	☐	☐	☒
c) Does the project have environmental effects which will cause substantial adverse effects on human beings, either directly or indirectly?	☐	☐	☐	☒

Discussion

Details are discussed in chapter 3.

APPENDIX B: An ecological risk analysis for the use of *Bombus impatiens* for pollination of field crops in California.

An Ecological Risk Analysis for the Use of *Bombus impatiens* for Pollination of Field Crops in California

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October 11, 2006

Executive Summary

A shortage of pollinators has been reported for California resulting at least partly from the infestation of honeybees with the parasitic mite, *Varroa destructor*. *Bombus impatiens* is a potential supplement to the native pollinators and honeybees. This risk assessment evaluates the potential risks resulting from the use of *B. impatiens* for pollination of field crops in California in agro-ecosystems south of the 39th parallel. The assessment focuses on the six species of the subgenus *Pyrobombus* that are closely related to *B. impatiens* and are native to California.

Unlike honey bees, bumblebees “buzz-pollinate” or sonicate flowers which provides better access to pollen and can make them more effective pollinators of some flowers. Bumblebees actively collect pollen, and therefore effectively pollinate crops such as tomatoes that do not produce nectar. Native bees such as bumblebees are more efficient at pollinating watermelons and other cucurbits.

Eight potential sources of risk are considered:

1. Transmission of parasites or pathogens to native organisms.
2. Competition with native flower visitors for floral resources.
3. Pollination of exotic weeds.
4. Changes in seed set of native plants (either increases or decreases).
5. Competition with native organisms for nest sites.
6. Genetic dilution of native bumblebee populations.
7. Escape of queens during shipping and in the field.
8. Escape of queens from improper disposal of hives.

Without mitigation, a potential for significant impacts to bumblebee species native to California exists from the introduction of *B. impatiens* for the pollination of field crops.

The necessary mitigations for each of the above potential risks are presented. The spread or introduction of diseases from commercial bumblebees will be adequately mitigated by careful rearing procedures and intensive monitoring of diseases and pests in the commercial colonies and rearing facility by Koppert and governmental agencies. Only colonies certified as disease-free are shipped. Competition between commercial bumblebees and native bumblebees will be mitigated by restricting the use of commercial colonies to those areas away from natural habitats where native bees thrive, and restricting the use of commercial colonies to the winter and spring when few native pollinators are active. To prevent or reduce impacts on native plants and to prevent the pollination of weeds, commercial colonies will only be placed away from native habitats; and colonies will only be allowed during the mid-winter/early spring. Use of commercial colonies for pollination of field crops only in the winter and spring will prevent most overlap in the seasons when native bees will be mating and will therefore mitigate gene dilution of native bumblebees. Few native plants are blooming at this time so the impacts on native plants also are reduced. Establishment of *B. impatiens* can only occur if queens escape, mate, successfully hibernate and establish nests the following year. Shipping containers with queen excluders and field procedures that will prevent damage to the colonies are sufficient to prevent the queens from escaping under almost all conceivable conditions. Growers are required to destroy and document the destruction of all colonies once they are removed from the field.

This assessment concludes that the mitigation procedures outlined are adequate to reduce the risk posed by the introduction of *B. impatiens* for pollination of field crops to a less than significant level.

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Introduction

Many plants, both crops and wild populations, are fully dependent on pollinators, and often specific pollinators, for seed production. When pollinators are lacking, or the plants they pollinate are missing, either or both will disappear (Biesmeijer *et al.* 2006). Bumblebees forage on a variety of floral resources, and most have longer tongues than honeybees allowing bumblebees to reach nectar even in deep tubular flowers. Bumblebees are able to continue foraging when light intensity is low, during light rain, and at temperatures ranging from 10 to 32°C. Consequently, many early season flowering plants benefit from the pollination services of bumblebees (Winter *et al.* 2006).

Pollination systems in many areas of agriculture are threatened by the inadequacy or lack of sustainable managed, native, or imported pollinators. Pollinator shortages can adversely affect crop production and commodity markets. Pollination deficits have been recorded in many parts of the world (Kevan and Phillips 2001). A shortage of pollinators has been reported for California as well, at least partly from the infestation of honeybees with the parasitic mite, *Varroa destructor* (Sousa 2005).

Bumblebees, including *Bombus impatiens*, are not affected by the *Varroa* mite (Greer 1999) and are a potential supplement to the native pollinators and honeybees. This risk assessment evaluates the potential risks resulting from the use of *B. impatiens* for pollination of field crops in California. The area of interest is the agro-ecosystems in 23 counties with sufficient non-grape agriculture south of the 39^{th} parallel (Figure 1) where the use of *B. impatiens* for the pollination of field crops is anticipated. No field uses north of the 39^{th} parallel or outside these 23 counties will be allowed.

Background

According to Thorp *et al.* (1983), 24 species of bumblebees (*Bombus* spp.) occur in California. The species proposed for use as a captive-reared pollinator of field crops is *B. impatiens* which is not native to California. *B. impatiens* is native to the entire east coast, west to Montana, Wyoming, Utah, and Arizona. It is active within this range from mid-April through mid-October (Griffin 1997)

Phylogeny of Bumblebees

The taxonomic organization of the genus *Bombus* includes 25 subgenera (Kawakita *et al.* 2004). Kawakita *et al.* (2004) list *B. impatiens* in the subgenus *Pyrobombus*. The following species of bumblebees of the subgenus *Pyrobombus* are native to California, *B. bifarius*, *B. caliginosus*, *B. centralis*, *B. edwardsii*, *B. flavifrons*, *B. huntii*, *B. melanopygus*, *B. mixtus*, *B. sitkensis*, *B. sylvicola*, *B. vandykei*, and *B. vosnesenskii* (Thorp *et al.* 1983; Kawakita *et al.* 2004). Of these species, those that are the most closely related to *B impatiens* appear to be *B. vosnesenskii*, *B. melanopygus*, *B. edwardsii*, *B. bifarius*, *B. huntii*, and *B. flavifrons*. Distributions of these species in California are depicted in Figures 2 – 7. Those species that overlap with agricultural regions where *B. impatiens* is proposed for field pollination in California include *B. vosnesenskii*, *B. bifarius*, *B. edwardsii*, and *B. flavifrons*. These same four species all occur within the permit request area south of the 39^{th} parallel.

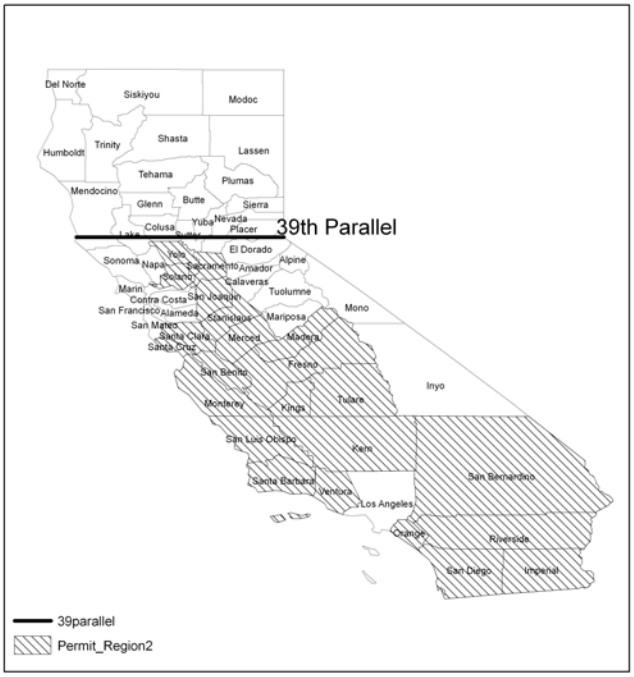

Figure 1. Area of California south of the 39th Parallel where introductions for field use of *B. impatiens* are being requested.

Figure 2. Counties with known locations of *B. vosnesenskii* throughout California based on Thorp *et al.* 1986.

Figure 3. Counties with known locations of *B. bifarius* throughout California based on Thorp *et al.* 1986.

Figure 4. Counties with known locations of *B. flavifrons* throughout California based on Thorp *et al.* 1986.

Figure 5. Counties with known locations of *B. huntii* throughout California based on Thorp *et al.* 1986.

Figure 6. Counties with known locations of *B. edwardsii* throughout California based on Thorp *et al.* 1986.

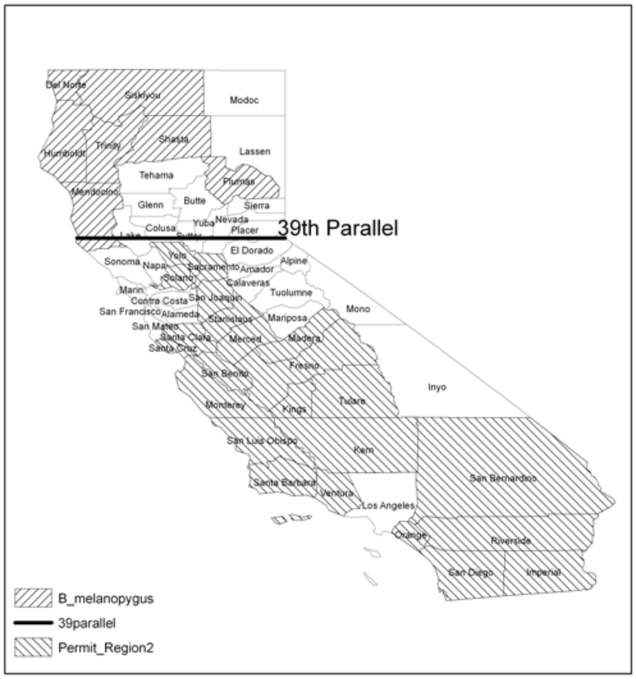

Figure 7. Counties with known locations of *B. melanopygus* throughout California based on Thorp *et al.* 1986.

The taxonomic status of *B. edwardsii* and *B. melanopygus* became uncertain when Owen and Plowright (1980) were unable to find any morphological differences between the two except for color dimorphism of the pile of the abdominal terga 2 and 3, and they discovered that this color dimorphism is controlled by a single gene locus with two alleles. They conclude that *B. edwardsii* and *B. melanopygus* are likely conspecific. To simplify the discussion for this risk analysis, the two will be considered separate despite the fact that only *B. melanopygus* is listed in a summary of the phylogeny of the subgenus *Pyrobombus* (Hines *et al*. 2006). Kearns and Thompson (2001) list *B. edwardsii* as a subspecies of *B. melanopygus*. The likelihood that *B edwardsii* is a subspecies of *B. melanopygus* is acknowledged, but since only *B. edwardsii* overlaps with the area anticipated for pollination of field crops by *B. impatiens*, the discussion will focus on *B. edwardsii*.

Annual Cycle of Bumblebees

Queen bumblebees emerge from hibernation in late winter or spring and search for a suitable nest site. Most species emerge gradually over several months (Goulson 2003a). The time of emergence of many species coincides with the flowering of willows (Alford 1975, Goulson 2003a, Kearns and Thompson 2001). *B. bifarius* emerges from hibernation early in the season, whereas under natural circumstances, *B. impatiens* emerges late. Information on the emergence time for *B. flavifrons*, *B. edwardsii*, and *B. vosnesenskii* was not available (Kearns and Thompson 2001), but activity periods are depicted in Thorp *et al.* (1983).

The species of interest, including *B. impatiens*, are all known to prefer to nest below ground except for *B. vosnesenskii* and *B. edwardsii* for which no information exists. During this time the queens forage for nectar and pollen as their reproductive organs complete their development (Kearns and Thompson 2001).

Once the queen has selected a nest site and has produced a wax cell in the nest, she will lay eggs. The time required from emergence from hibernation to laying the first eggs has not been described for all species in nature, but during commercial rearing under optimal conditions, approximately 60% of queens laying their first eggs within 14 days (Koppert Biological unpubl. data). However, development of a bumblebee worker through its four instars requires approximately 5 weeks (Alford 1975). The colony grows until the production of males and then queens occurs. Typically, batches of males and workers are reared together possibly followed by batches of all males. Males are produced when the queen lays unfertilized eggs (Alford 1975). Subsequently, batches of males and queens are followed by batches of purely queens (Alford 1975). Queens prevent female offspring from developing into queens by producing a specific pheromone. When the queen stops producing this pheromone, the females will develop into queens as long as they receive sufficient food during development (Goulson 2003a).

Most males leave the nest when they are between two and four days old and never return. They generally live for three or four weeks (Alford 1975).

Queens become fertilized when they are young, making a mating flight when they are about 5 days old (Alford 1975) usually between August and October (Greer 1999). Newly mated queens will begin hibernation at that time if they are ready, otherwise they will return to the nest. If ready, fertilized queens may enter their winter hibernation cavity in the soil as early as summer and remain there for up to nine months (Alford 1975). Only the fertilized queens live through the winter. The rest of the colony dies prior to winter.

Bumblebees as Pollinators

Bumblebees visit a wide assortment of flowering plants (Delaplane and Mayer 2000, Thorp *et al* 2002, Kremen *et al.* 2002b). Bumblebees visit flowers to collect both pollen and nectar (Thorp *et al.* 2002). Unlike honey bees, bumblebees "buzz pollinate" or sonicate flowers which provides access to pollen the bees would not otherwise be able to access (Gray and Leong 2003, Thomson and Goodell 2001). Bumblebees work at cooler temperatures than honey bees (Greer 1999).

Some crops such as tomatoes do not produce nectar, so honey bees will not visit tomato flowers. However, since bumblebees actively collect pollen, bumblebees readily visit such crops. Native bees such as bumblebees are more efficient at pollinating watermelons and other cucurbits because they will transfer greater amounts of pollen in a single visit than will honey bees (Xerces Society Fact Sheets, Stanghellini *et al*. 2002). In a time when honey bee availability is diminishing, bumblebees were found to be a viable replacement pollinator for avocado trees (McNeil and Paddock 2003). Bumblebees deposited more pollen on apple stigmas than honey bees, but the transfer rate was similar between bumblebees and honey bees for almonds (Thomson and Goodell 2001). Bumblebees will not adequately pollinate all crops, but many crops such as red clover (Thorp 2003) are effectively pollinated by bumblebees.

Potential Sources of Risk

Goulson (2003b) lists five undesirable effects exotic bees could have on ecosystems. These are:

1. Transmission of parasites or pathogens to native organisms.
2. Competition with native flower visitors for floral resources.
3. Pollination of exotic weeds.
4. Changes in seed set of native plants (either increases or decreases).
5. Competition with native organisms for nest sites.

Three additional potential risks are added in this analysis because the intent of using *B. impatiens* on field crops is to prevent its establishment. These additional risks are (Thorp, pers. comm.):

6. Genetic dilution of native bumblebee populations.
7. Escape of queens during shipping and in the field.
8. Escape of queens from improper disposal of hives.

This list of eight potential risks can be divided into two general scenarios. Items 1 – 4 and 6 on the list could potentially occur while *B. impatiens* workers perform field pollination or by males foraging from their commercial hives. Items 5, 7, and 8 would potentially occur only after *B. impatiens* has become established, and queens are free-living in the wild.

Potential Risks Regardless of Establishment

Disease/Parasites

A principal concern regarding the introduction of an exotic insect is the exposure of closely related native species to new diseases (Thorp 2003). According to Goulson (2003a), the rate of exotic pathogens infecting native bee populations is not well known since so few studies have been done. He suggests if the native bees are suitable hosts, an epizootic would likely go unnoticed. In this particular scenario, where the species for introduction is native to North America, the discussion here will focus not on the introduction of new diseases, but increasing the incidence of existing diseases. We will make no attempt here to fully describe the effects related to infection with these various pathogens or parasites. We recognize that disease transmission is of great concern and will discuss how transmission could occur and the likelihood of using *B. impatiens* for pollination of field crops leading to transmission of disease.

Morandin *et al.* (2001) have shown that the loss of worker bumblebees can be high from greenhouses in Ontario that grow tomatoes. Colla *et al.* (2006) sampled bumblebees in the vicinity of greenhouses and away from greenhouses in Ontario and found a higher incidence of *Crithidia bombi* and *Nosema bombi* near the greenhouses. Since others have found a higher incidence of some diseases in commercial bumblebee colonies, they concluded the source of the higher incidence was the commercial bumblebees in the greenhouses. The possibility that commercial bumblebees from a greenhouse could increase the incidence of disease in bumblebees outside the greenhouse stresses the need for disease-free bumblebees for use in field pollination.

Introduction of new strains of existing diseases or parasites into a region can be particularly harmful to colonies that have previously not been exposed. Schmid-Hempel (2001) has shown that the interaction between host and parasites is a continual process of co-evolution. Parasites adapt over time to defeat the defenses of host population and become more virulent from one generation to the next. Also, a naive host population can be particularly susceptible to a new parasite, and could be more severely impacted than a population that has adapted to routine exposure (Schmid-Hempel 2001). Genetics also affect resistance to parasites. Different offspring from queens mated to males of differing genetic stock showed differences in their susceptibility to *Crithidia* in laboratory trials (Baer and Schmid-Hempel 2003).

Table 1. Parasites and pathogens reported in bumblebees.

Taxonomic Group	Parasite	Remarks
Virus	Acute Bee Paralysis virus Entomopox virus*	Uncertain status in nature
Bacteria	*Spiroplasma** *Aerobacter cloaca* Other unidentified bacteria	In hemolymph
Fungi	*Acrostalagmus* *Beauveria bassiana** *Candida** *Hirsutella**, *Metarhizium**, *Paecilomyces**	Possibly shortens hibernation
Protozoa	*Apicystis bombi** *Crithidia bombi** *Nosema bombi** *Neogregarina* sp.	Can completely destroy the fat body Can kill entire colonies but highly variable in expression. A creeping disease
Nematodes	*Sphaerularia bombi**	Infects hibernating queens and castrates them. Known from almost all species.
Hymenopteran Parasitoids	*Syntretus* sp.,* *S. splendidus* *Melittobia acasta, M. chalybii* *Monodontomerus montivagus* *Pediobius williamsoni*	Probably attacks spring queen exclusively.
Dipteran Parasitoids	*Apocephalus borealis* *Boettcharia litorosa** *Helicbia morionella** *Brachioma devia, B. sarcophgina, B. setosa* *Conops algirus, C. argentifacies, C. elegans, C. flavipes, C. quadrifasciatus, C. vesicularis* *Melaloncha* sp. *Physocephala* spp.,* (*P. brugessi, P. dimidiatipennis* *P. dorsalis, P. nigra, P. obscura, P. rufipes* *P. sagittaria, P. tibialis, P. vittata*) *Senotainia* sp.,**S. tricuspis* *Sicus ferrugineus* *Zodion* sp.*	Feeds on thoracic muscles Can be extremely destructive Investigated , particularly in *B. terrestris* Pupa can be hyper-parasitized by pteromalid wasps Investigated , particularly in *B. terrestris*
Lepidoptera	*Ephestia kühniella*	Feeds on provisions
Acari	A large number of species	Unclear status as parasites

Source: Schmid-Hempel 1998
* Confirmed to exist in North America (Macfarlane *et al.* 1995)

Bumblebees are host to numerous pathogens and parasites (Table 1). In addition to those listed in Table 1, additional pests known to infest bumblebees in North American include: fungi-*Verticillium lecanii, Aspergillus candidus;* mites-*Locustacurus buchneri;* and flies-*Sarcophaga sarrcenoides* (Macfarlane *et al.* 1995). Bumblebees are also host to a large number of mites.

Most mites are probably not truly parasitic, but live on the debris in the nest or consume some of the nest provisions. However, at least one species, the tracheal mite (*Locustacarus buchneri*), can be extremely damaging for the affected individual and may put the entire colony at risk (Schmid-Hempel 2001). Also, disease organisms such as *Nosema*, *Apicystis*, *Crithidia* spp., and *Locustacrus buchneri* are difficult to detect (Thorp 2003). Although Winter et al. (2006) cite a number of studies where *Crithidia* were present in commercial stocks (*e.g.* Gegear *et al.* 2005, 2006; Otterstattter *et al.* 2005); none of these stocks of *B. impatiens* were provided by Koppert (Michael Otterstatter pers. comm.).

Macfarlane *et al.* (1995) suggest that trade in bumblebee colonies between eastern and western North America could spread minor enemies of bumblebees. Thorp (2003) also raises concerns regarding diseases of species outside their natural ranges that apply to the introduction of *B. impatiens* from eastern North America to California. He states:

> Not enough is known about the potential variation in virulence of bumblebee diseases, especially related to interspecific interactions, so that what may seem a rather benign pathogen in one species may be highly infectious and/or deadly in other species.

Individual bees do not need to come into actual contact with infected individuals or enter foreign colonies to become infected with some diseases. Some mites will leave an infested bee at a flower to wait for another bee to visit that same flower. The mites will then infest the new bee. *Parasitellus* mites can survive on flowers for up to 24 hrs (Schwarz and Huck 1997).

The introduction of *B. impatiens* into California is unlikely to impact existing naturalized or commercial honeybee populations. Most diseases of honeybees and bumblebees do not appear to be cross-infective. For example, two species of *Nosema* exist. Although *Nosema apis* is a pathogen for honeybees, it does not infect bumblebees, and *Nosema bombi* does not infect honeybees. However, one exception to this rule is the small hive beetle (*Aethina tumida*). Commercial bumblebee colonies placed close to infested honeybee hives became infested with and experienced successful reproduction of small hive beetles in the field (Spiewok and Neumann 2006) indicating a potential for a spread of small hive beetles from honey bees to commercial or wild bumblebees in the field. It would therefore also be possible for small hive beetles to spread from infected commercial bumblebees to native bumblebees. Another recently reported exception appears to be the deformed wing virus previously restricted to honeybees. The deformed wing virus has recently been identified in commercial *B. terrestris* and wild *B. pascuorum* in Europe. In honeybees, this virus is often associated with the mite *Varroa destructor*, but no mites were associated with the infected bumblebees (Genersch *et al.* 2006).

Concerns over disease spread between *B. impatiens* and native bees are appropriate. In addition, a legitimate alternative question is whether the potential for disease to spread from commercial to native bumblebee species will pose a greater or lesser risk than the existing risk of disease spreading from honeybees to native bumblebees? Since no restrictions are placed on the use of honeybees, no controls are in place to prevent small hive beetles or deformed wing virus or other diseases in honeybees from infecting native bumblebees. Where commercial bumblebees are a viable alternate pollinator, there might be an actual reduction in risk to native bees from the use of commercial bumblebees as compared to the current use of honeybees.

Without effective mitigation, a potential risk exists for the transfer of disease from commercial bumblebees to native bumblebees. This risk will depend on the prevalence of disease or pests that infest commercial bumblebees as well as the extent to which commercial bumblebees interact with native bumblebees.

Competition for Pollen and Nectar with Native Bumblebees

Sols (1987) documents how queen bumblebees in California will be displaced when queens of different species are introduced. Although competition among queens for pollen and nectar is of little concern in this assessment, this is the only available information for competition among different species of bumblebees in California. It is possible that worker *B. impatiens* will compete with queens of other species that emerge from hibernation after the commercial colonies of *B. impatiens* are present. Competition for pollen and nectar could also occur among workers of those native bumblebee species with activity periods for workers that overlap with the period when worker *B. impatiens* will be present (Table 2).

As worker *B. impatiens* collect pollen and nectar, that resource will become unavailable to native pollinators. The risk of *B. impatiens* workers removing pollen or nectar that would otherwise be collected by native pollinators is low. Koppert Biological Systems, Inc. will limit the availability of commercial *B. impatiens* to only mid-winter and early spring (prior to week 22, approximately the first week in May) before most native pollinators are active. Only *B. edwardsii* workers will be active in high numbers during this period (Table 2).

Table 2. Activity periods for worker bumblebees.

Species	Worker Activity Period	Worker Peak Activity
*B. impatiens**	Late January – Mid-May	Mid-February – Late April
B. bifarius	Early May – Mid-September	Early June – Mid-August
B. edwardsii	Mid-January – Late August	Late February – Late June
B. flavifrons	Early May – Late September	Early June – Mid August
B. vosnesenskii	Mid-January – Mid-October	Early June – Late July

* Anticipated according to projected commercial use patterns
Source: Thorp *et al.* 1983

Native bees often do not thrive in agroecosystems (Kearns and Thomson 2001). *B. impatiens* colonies will only be situated in crops during the period immediately preceding or during the period when blooms are present requiring pollination, not in more natural areas where native bumblebees will more likely be present. For these reasons, native bees are unlikely to be impacted by the temporary supplementation of pollination services by *B. impatiens* focused on high production agricultural areas. The intention of using commercially-reared bumblebees for pollination of field crops is to supplement the pollination services of native pollinators when those pollination services are insufficient to meet a grower’s needs.

B. impatiens shows little tendency to move forage far from their colony. Within a fragmented area broken up by roads, railroads, and other human-made features, *B. impatiens* showed a surprising reluctance to move from one habitat patch to another on the opposite side of one of these features (Bhattacharya *et al.* 2003). Should farm roads, equipment lots, etc. produce a similar response, *B. impatiens* used for pollination in field crops might have a strong tendency to remain within the field boundaries, especially if there are sufficient resources within the field to meet their needs. The tendency for *B. impatiens* to remain close to the colony and within the intended crop is supported by work in blueberries where the median value of 99.7% of pollen loads consisted of blueberry pollen despite the presence of other flower crops nearby (Whidden 1996).

Concerns over competition between *B. impatiens* and native bees are appropriate. A legitimate alternative question is whether the competition among bumblebee species will be any greater than the existing competition between native bumblebees and honeybees. Forup and Memmott (2005) have shown that there is a negative relationship between the numbers of honeybees and the number of bumblebees present in native habitats. They were not able to determine whether the honeybees caused the reduction in bumblebee numbers or whether the honeybees were benefiting from a lack of bumblebees. Their work indicates that there is an existing interaction between honeybees and bumblebees, so the introduction of *B. impatiens* might be no worse regarding competition for flower resources than the existing competition with honeybees. This dynamic might change if *B. impatiens* were to be permanently established. If *B. impatiens* becomes established in native habitats away from agro-ecosystems, competition with native bumblebees could increase.

The risk of competition for pollen and nectar between *B. impatiens* and native bumblebees even without mitigation is less than significant. Since *B. impatiens* is unlikely to forage far from the colony, and colonies will be placed in agricultural fields or orchards when the crop is in bloom, there will be little opportunity for *B. impatiens* to collect pollen or nectar such that native bumblebees would therefore go without necessary resources.

Impacts on Native Plants

Impacts on pollination of native plants might be limited since introduced species often focus on introduced plants (Donovan 1980) so there could be limited reduction in reproduction of native plants. Native bumblebees depend on a succession of pollen producing species throughout the season because colonies are active for many months (Thorp *et al.* 2002). However, the period that *B. impatiens* will be present in crops will be restricted to five weeks in California's mid-winter/early spring before most native pollinators are active. Placing the colonies in agricultural fields or orchards when crops are in bloom will limit the need for *B. impatiens* to forage on native plants. Therefore, the risk to native plants even without any mitigation will be less than significant.

Pollination of Exotic Weeds

In areas where exotic plants native to the same region as *B. impatiens* are present, it is possible that *B. impatiens* will selectively pollinate those flowers enhancing the invasiveness of the undesirable weeds (Thorp 20003). However, only early blooming plants will be pollinated since the *B. impatiens* colonies will be removed from the field by the late spring. Since *B. impatiens* are not currently free-ranging in California, it is not clear what exotic weeds they might select, but some level of risk does remain that they could actively pollinate early-blooming exotic weeds.

Genetic Dilution of Native Bumblebees

Two routes exist that exotic genes can enter native bumblebee gene pools. Males could breed with queens of closely related species or worker bees could enter local colonies and become drone layers (Thorp 2003). The peak activity periods for male bumblebees of the species of interest range from Early April to Mid-September (Table 3) and *B. impatiens* peak activities will occur from Mid-February to Late April. The activity period for queens (Table 4) is longer than that of males, but part of that activity period is when queens are first establishing their colonies. Queens of some species, such as *B. vosnesenskii*, have a bimodal activity peak (Thorp *et al.* 1983) with the second peak coinciding with the peak activity of males. This suggests the second activity peak constitutes the young queens out in search of mates. These activity periods are for the entire state of California, so early season male activity could be restricted only to some areas. However, from the available information, those regions are not apparent.

The time during which commercial colonies could be present in the field plus the subsequent three to four week period during which males from the colonies might still be alive would overlap the entire activity periods for males of all four species in Table 3, but would only overlap the peak activity period for *B. edwardsii* males. There would also be some overlap with the second peak of activity of queen *B. edwardsii* demonstrating some potential risk for interbreeding between male *B. impatiens* and *B. edwardsii* queens (see Table 4). The level of this potential risk will depend partly on whether the regions where *B. edwardsii* queens are active early overlap with agricultural regions where *B. impatiens* will be used for field pollination of crops.

Table 3. Activity periods for male bumblebees.

Species	Male Activity Period	Male Peak Activity
*B. impatiens**	Late January – Early June	Mid-February – Late April
B. bifarius	Mid-May – Early October	Mid-July – Mid-September
B. edwardsii	Early February – Mid-September	Early April – Late June
B. flavifrons	Early May – Mid-September	Mid-July – Mid-August
B. vosnesenskii	Mid-February – Early November	Early June – Mid-September

* Anticipated according to projected commercial use patterns
Source: Thorp *et al.* 1983

Koppert Biological Systems has attempted to interbreed four native bumblebees with *B. impatiens* (unpublished data). Attempts to cross *B. impatiens* with *B. occidentalis*, *B. vosnesenskii*, *B. affinis*, and *B. bimaculatis* all resulted in no mating or no viable offspring. Of these species, *B. vosnesenskii* and *B. bimaculatis* are in the subgenus *Pyrobombus* along with *B. impatiens*. These failed attempts to hybridize among a number of species with the subgenus *Pyrobombus* suggest that interbreeding within the subgenus might be unlikely.

Potential Risks Resulting in or from Establishment

Escape of Queens

Regardless of how queens escape from commercial colonies, there is precedence that bumblebees can quickly become established. Within nine years of when they were first observed, bumblebees (*B. terrestris*) have come to occupy the entire island of Tasmania, including many native habitats (Hingston *et al.* 2002). Many conditions would need to be met before *B. impatiens* might become that extensively established in California, but the example of *B. terrestris* in Tasmania indicates considerable effort needs to be made to prevent the escape of *B. impatiens* queens in California.

Escape During Shipment

Accidents can occur during the shipping process, so the possibility exists that packaging could be damaged and the queen from the colony could escape. Therefore, there is a risk that colonies could be destroyed during shipment, queens could escape and *B. impatiens* could become established in California.

Escape *In Situ*

While commercial hives are in the field, the hives could be damaged by vandalism, accidentally by farm workers/farm equipment, or wildlife. Experience in eastern North America has shown that the hives within the QUAD shipping box can withstand "break-in" attempts by all but the largest of animals. Medium-sized animals such as skunks (*Mephitis* spp.) or raccoons (*Procyon lotor*) are unable to gain entry into the hives. Only large mammals such as black bears (*Ursus americanus*) have been able to gain access to the hives and could potentially allow queens to escape (Koppert unpubl. data). Therefore, a potential risk exists that a colony could be damaged while in the field and queens could escape.

Escape During Disposal

Following the allotted time for pollination in the field crops, the commercial colonies will need to be disposed. Should any queens produced prior to or during the time the colony is in the field survive the disposal process; they could possibly be released along with any males in the colony. Therefore, a risk exists that queens could exist during colony disposal.

Competition for Nests

Queens that emerge late from hibernation or have their nests destroyed will sometimes take up residence in existing nests, even of other species of bumblebees (Kearns and Thomson 2001). Should a commercial colony be destroyed while in the field or improperly disposed and the colony queen survives, the *B. impatiens* queen could attempt to usurp the nest of a native queen. The likelihood of this occurring is currently unknown. Since *B. impatiens* is not native to California, how queens might behave under the conditions in California during mid-winter/early spring are not known.

The probability of an introduced *B. impatiens* queen (*e.g.*, one bought and shipped in, not emerging from hibernation) successfully usurping a native bumblebee nest will be dependent on the likelihood of a *B. impatiens* queen escaping from the commercial colony, the likelihood of finding a native bumblebee nest, and the likelihood of the *B. impatiens* queen being able to "defeat" the native queen. Since queens for all the species of interest (Table 4) are active in mid-winter/early spring, it would possible that active nests would be present. However, ground nesting bees such as *B. impatiens* require a patch of undisturbed soil in a sunny spot (Greer 1999). In many agricultural settings, such a location is rare (Kremen *et al.* 2002a). It seems more likely that any colony-founding queen that escapes from an active commercial colony will return to the commercial colony. A newly produced queen would start foraging and looking for mates, prior to hibernation and would not usurp an existing native nest.

Table 4. Activity periods for queen bumblebees.

Species	Queen Activity Period	Queen Peak Activity
*B. impatiens**	None (unless escapes occur)	None (unless escapes occur)
B. bifarius	Mid-March – Mid-September	Early June – Mid July
B. edwardsii	Year-round	Early February – Early July
B. flavifrons	Mid-April – Mid-August	Early June – Early July
B. vosnesenskii	Year-round	Mid-February – Early July

* Anticipated according to projected commercial use patterns
Source: Thorp *et al.* 1983

The use of queen excluders has been highly successful at preventing escape of queens from colonies. Despite the shipment into CA of an average of about 2300 hives in the years 2003 – 2005 for pollination in greenhouses, mainly in Ventura County, there has been no reported establishment of *B. impatiens* in the areas around those greenhouses (Winter *et al*. 2006). Morandin *et al.* (2001) report that worker bumblebees in greenhouses readily exit and return through vents, so there is no reason to expect that if queen excluders had a notable failure rate and conditions in CA were suitable, queens could leave the greenhouse, mate, hibernate and establish colonies.

The more likely route by which queens would escape the colony is from the destruction of the colony during shipping, in the field, or during disposal (see later discussions). Although these routes are more likely than a queen getting past a queen excluder, the likelihood remains quite low. However, the potential for queens escaping appears real since Mackenzie (pers. comm. in Winter *et al.* 2006) reports that commercial queens have been sighted near greenhouses in the Frazer Valley of British Columbia. The risk of a queen finding a native bumblebee nest is low since only *B. edwardsii* and *B. vosnesenskii* will be active when colonies are used for pollination and *B. impatiens* colonies will only be used in high production agricultural settings where native bumblebee nests are rare. Since there are no known occurrences of a *B. impatiens* queen attempting to usurp a nest from one of the native California bumblebees, the likelihood of success is unknown.

The other potential route to competition for nests would be for a queen to escape, hibernate through the winter, emerge the following spring and then use an existing underground nesting location. The same hurdles are in place for the queen escaping as were discussed above. Additionally, the *B. impatiens* queen must also acquire sufficient reserves to survive hibernation, must locate a suitable hibernation site, and must survive the hibernation and experience suitable conditions to induce ovary development, normally soil temperatures below freezing (Szabo and Pengelly 1973). It is during hibernation that the corpora allata releases gonadotrophic hormones required for the maturation of ovaries (Alford 1975). Therefore, undergoing hibernation successfully is a critical and necessary process for the maturation of a queen. Experience rearing *B. impatiens* has shown that the minimum and maximum periods the queen must hibernate are 8 weeks and 26 weeks, respectively (Koppert unpubl data). Therefore, a young queen from an imported colony would need to emerge from hibernation no later than approximately 6 mo. after entering hibernation. She would be emerging in late fall or early winter, before there would be many if any flowers available for her to gather pollen and establish a colony.

Wild *B. impatiens* queens often hibernate close to their nests (Alford 1975). Assuming an escaped young queen would do likewise, the queen would need to locate a suitable hibernaculum in or near the agricultural area where the commercial colony was used for crop pollination. Depending on the intensity of agriculture in the region, this could be difficult (Kremen *et al.* 2002a). There is no information on the success rate of *B. impatiens* hibernating in California since it has never been reported and to our knowledge has never occurred.

The conditions in the agricultural regions of California are quite different from the conditions *B. impatiens* experiences in its native range in eastern North America where there are prolonged periods during the winter near or below freezing. Therefore, the risk for *B. impatiens* queens to successfully hibernate and establish colonies in California is low.

Mitigation Measures

Mitigation measures are only useful if shown to be effective. The preceding discussion acknowledges there are potential risks related to the use of *B. impatiens* for pollination of field crops in California. To help ensure that the following mitigation measures are adequate, Koppert will work with the California Department of Food and Agriculture and others to develop protocols for assessing the environmental impacts of the use of *B. impatiens* for field crop pollination. All growers will be required by Koppert to sign contracts that stipulate the handling requirements and the duration the *B. impatiens* colonies can remain in the field prior to receiving any bumblebee shipments.

Measures to Prevent Risks Not Associated with Establishment

Measures to Prevent Spread of Disease/Parasites

Koppert operates a single commercial rearing facility near Detroit, MI. A single centralized rearing facility enables Koppert to maintain strict control over the health of the bumblebees used for brood stock and being shipped. Koppert maintains a stock of *B. impatiens* that has demonstrated superior disease resistance properties (as opposed to *B. occidentalis* that proved susceptible to disease under commercial rearing conditions).

Koppert currently has rigorous disease/parasite detection and prevention procedures in place. Hives are routinely monitored by Koppert’s Quality Control Department and inspected every other week by the Michigan Department of Agriculture. These checks and inspections determine whether the protozoan parasite *Nosema bombi*, the parasitic nematode *Sphaerularia bombi*, the tracheal mite *Bombacarus buchneri* and all other internal and external mites, and various flagellates and *Bacillus* strains are present. Inspections also check for the following pests: wax moths, hive beetles, meal moths, *Drosophila* spp., and *Melittobia* spp.

With the increase in the breeding population there is no longer a need to bring in genetically fresh material from the wild, hence Koppert rarely introduces new brood stock to its rearing facility. The last time was 4 years ago. No introductions are anticipated to be needed. In the rare occasion that stock is brought in, it is kept in quarantine for a minimum period of 6 months, or until any pest or disease issues would have become apparent. Also queens at Koppert's rearing facility hibernate outside of the soil and do not come into contact with soil during hibernation. This effectively prevents developed production hives becoming infected with the soil dwelling nematode, *S. bombi*. *Nosema bombi* has never been detected in Koppert's production facility. Koppert will continue to implement the most sensitive, practical disease and prevention procedures. For example, Koppert is willing to implement a trial of the genetic probe using a polymerase chain reaction of partial rRNA sequence to detect *Nosema* (Klee *et al.* 2006) if this is deemed necessary. This has been shown to be more sensitive than light microscope procedures. Only bumblebees that have been certified as disease and parasite free by the Michigan Department of Agriculture will be shipped to California.

Measures to Prevent Competition with Native Bumblebees

Commercial colonies will be placed away from natural areas reducing the potential that any worker *B. impatiens* might enter a wild bumblebee nest and take up residence, if this is even possible. Commercial colonies will only be allowed in the field for five weeks and will only be available for growers to place in their fields during mid-winter/early spring (weeks 3 to 22, or Late January to Early May). By limiting the time the *B. impatiens* is present in the field and by limiting the timing to before when many native pollinators become active in large numbers, the direct competition for resources with native bumblebees will be greatly reduced.

With the introduction of captive-reared bumblebees, it has become more apparent to growers that native bees (introduced or not) have a tremendous value. Through education and outreach, Koppert is working to encourage the propagation of wild bumblebees in combination with giving advice on the proper mix of pollinators to introduce. Koppert is using the term 'integrated pollination management' (IPM) as a tool to create awareness with growers regarding the value of native pollinators. Also on the west coast, where feasible, Koppert will provide suggestions on farm management practices that will be friendly to native, wild pollinators, including bumblebees. Koppert will encourage the use of *B. impatiens* only in areas of intensive agriculture, away from areas of high densities of native bumblebees and other natural pollinators.

Measures to Prevent Impacts on Native Plants and Prevent Pollination of Exotic Weeds

Two principal mitigation measures will be implemented to prevent or reduce impacts on native plants and prevent pollination of exotic weeds: 1) commercial colonies will only be placed in the interior of crop fields or orchards; and 2) colonies will only be allowed during mid-winter/early spring. Placing the colonies only away from native habitats will reduce the likelihood that *B. impatiens* workers will need to travel beyond the field or orchard borders. By limiting the time the commercial colonies are in the field, the potential availability of flowering native plants or weeds will be reduced.

Measures to Prevent Gene Dilution of Native Bumblebees

The season when *B. impatiens* will be allowed for field pollination is being selected specifically to minimize overlapping with the breeding seasons of native bumblebees of the subgenus *Pyrobombus*, those bumblebees most closely related to *B. impatiens*. By the time queens of native bumblebees of the genus *Pyrobombus* are out in search of mates, few if any *B. impatiens* males will remain to possibly mate with them. Also, the *B. impatiens* colonies will only be allowed on highly agricultural regions away from native habitats where native bumblebees are prevalent.

Koppert is currently investigating species native to California for use in commercial rearing, but has not perfected the rearing techniques to allow for reliable commercial production and availability. To meet the current pollination needs, *B. impatiens* is being proposed for temporary use for field pollination of crops in California. Once a native bumblebee from California has been developed for reliable commercial production, *B. impatiens* will no longer be used for field pollination of crops in California.

Since the one species of native bumblebees that is likely to be active during the time when *B. impatiens* is being considered for field pollination is *B. edwardsii*, Koppert will attempt to interbreed male *B. edwardsii* with queen *B. impatiens*. If interbreeding proves to be unsuccessful, no immediate monitoring of sites where *B. impatiens* is used for field pollination will be required. If interbreeding is successful at producing viable offspring, areas where *B. impatiens* is to be used for field pollination will be monitored for the presence of *B. edwardsii*. If *B. edwardsii* are present, no *B. impatiens* will used for field pollination in that area.

Measures to Prevent Establishment and Its Impacts

A critical mitigating measure to prevent the establishment of *B. impatiens* in California is the restriction of use south the 39th parallel and only in highly agricultural regions such as the Sacramento, San Joaquin Valleys and areas of southern California. Areas predominated by native habitats where native pollinators thrive will be avoided. The intent of using *B. impatiens* for pollination of field crops in California is to supplement pollination by honeybees or other native pollinators, not to replace those other pollinators. Therefore, the use of *B. impatiens* will only be encouraged where it is truly needed within these areas.

Measures to Prevent Escape of Queens

The sturdy packaging used during shipping will prevent all but the most severe mishaps from causing sufficient damage that queens might escape. The QUAD boxes have undergone considerable testing to assess their reliability. Koppert has dropped them off the roof of a two story building. The box survived intact. In one instance during actual shipping, two quads fell off a truck. Again, the boxes remained intact. During the past years Koppert has shipped QUAD along the U.S. east coast to smaller growers as well. In these cases, United Parcel Service (UPS) is often used as a delivery system. UPS employs a shipping system in which all their boxes (and the QUAD) roll over conveyer systems in distribution facilities and are handled multiple times prior to arriving at the final destination. Even during using this system with excessive handling, no bees have been reported to have escaped from the QUAD.

Koppert uses a multiple layer secure shipping package. The bumblebee colony is housed in a secure plastic hive (Figures 7 & 8). This same hive is used during colony rearing. The plastic hive is placed within a sturdy cardboard box, similar to a "banker's box" (Figure 9). A plastic sliding door can be opened to allow bumblebees to leave the hive. Four hives fit snugly into a "QUAD" shipping box constructed out of sturdy corrugated plastic (Figure 10) which is then securely taped shut. The QUAD is also used for placement of the hives in the crop for pollination.

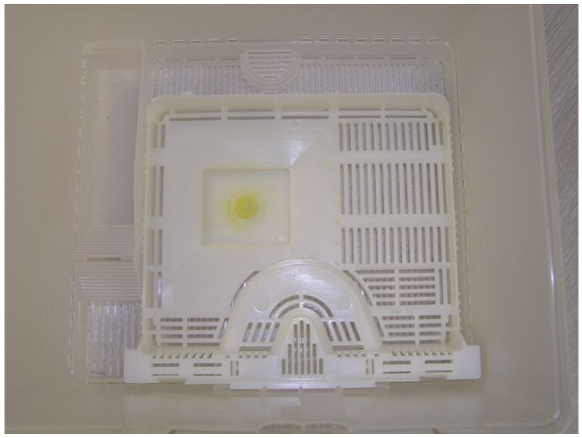

Figure 8. Hive plate that snaps into nest box.

Figure 9. Plastic nest box.

Figure 10. Cardboard box for housing individual hives. Hive exit is at top center with queen excluder in place.

Figure 11. Thick corrugated plastic outer shipping packaging. The open flap on the right indicates one of the four hive exists.

Growers will be required to place the colonies only within close proximity (*i.e.*, within three feet) of a protective, large tree, or other large structure that will prevent accidental disturbance by farm equipment. In the case of placement of QUAD in black bear areas growers have employed electric fencing to surround the QUAD successfully preventing bear from disrupting the hives. A pollination contract will be established between each grower and Koppert noting the prerequisites (mitigations) necessary to be followed prior to being eligible to receive *B. impatiens* colonies. A proposed pollination contract is included as an APPENDIX.

Colony Destruction

After providing pollination services for no more than five weeks in the field crop, each *B. impatiens* colony will be destroyed and disposed. Approved methods for colony destruction are either by freezing or downing. Koppert will provide complete details on how this is to be performed along with each shipment. Once the colony has been destroyed, the grower will notify Koppert or its distributor how the colony(ies) was(were) destroyed and disposed and the dates on which these actions took place.

Measures to Prevent Competition for Nests

No additional mitigation measures are anticipated to prevent competition for nests. Those mitigations to prevent the escape of queens and the establishment of colonies are expected to be adequate to prevent competition for nest sites between *B. impatiens* and native bumblebees.

Risk Determination

The ecological risks that exist regarding the introduction of *B. impatiens* into California south of the 39th parallel pertain to spread or increase in disease or pest pressure, competition for resources regardless of whether *B. impatiens* becomes established in California, and finally the establishment of *B. impatiens* in California. The risk of increase or spread of disease and pests is considered less than significant after the mitigations described for the following reasons: 1) Koppert Biological currently maintains a certified disease/pest free rearing facility, 2) Koppert Biological continues to incorporate up-to-date, industry-leading screening and prevention methods, and 3) as required, Koppert Biological will instigate a trial genetic probe screening program to test whether its current screening methods are adequate. The risk of competition for resources is considered less than significant following the mitigations described for the following reasons: 1) *B. impatiens* will be allowed in the field only from mid-winter to late spring which is earlier than most native bumblebees become active, and 2) *B. impatiens* will be allowed only in highly agricultural areas away from native habitats where native bumblebees are prevalent. The risk of establishment is less than significant after the mitigations described for the following reasons: 1) queen excluders have proven effective at preventing the escape of queens under normal circumstances, 2) Koppert Biological packaging materials have proven resistant to destruction from all but the most severe impacts, 3) handling procedures have proven adequate to prevent mishaps, 4) agro-ecosystems have proven unfavorable habitats for bumblebees, and 5) proper disposal of the colonies after their field use is terminated will prevent young queens from remaining in the wild after the colony has been removed from the field.

The final risk determination is that the introduction of *B. impatiens* for the pollination of field crops, following the mitigation measures described in this assessment will pose a less than significant risk to the environment of California.

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APPENDIX C: Disease management procedures

Hive Selection and Quarantine

Hives selected and transferred into Phase 4 breeding are the basis for all offspring used for sales and continuous breeding. It is imperative that proper screening and testing be conducted before any hive enters the breeding process. Every hive selected for transfer into Phase 4 for queen and male production must be tested for disease / pests before selections occur. Each hive should be examined for physical symptoms of disease such as high mortality, diarrhea, lethargy, aborted larvae or pupae, deformities, and foul smelling brood. Nests should also be inspected for parasites such as hive beetles, flies, parasitic wasps (*Melittobia*), and wax moths. Hives selected for transfer must remain in a segregated unit away from the main breeding until dissection test results are received. No bees may be removed from these hives until they receive clearance from the QC department.

Samples for Dissection

All hives that meet guidelines for transfer to Phase 4 should have 10% (5-10 workers minimum) of the population removed for testing. Pull bees to be dissected and place them in a sample cup. Label the hive and corresponding cup with the date, breeding line, and sample #. Place samples in the freezer for 8 hours before dissecting. Every hive selected for Phase 4 must undergo testing for the following parasites / pathogens:

Nosema bombi
Chrithida bombi
Sphaerularia bombi
Parasitellus (external mites)
Bombacarus buchneri (and other tracheal mites)

Any hive that tests positive for one the aforementioned parasites should be discontinued immediately and Management must be notified. All remaining hives from the group that had good results shall continue to be quarantined while secondary tests are conducted to confirm their cleanliness.
Test results from every group are to remain on record for at least one year and should be made available to the Michigan department of Agriculture inspector at all times. Hives shall remain labeled with sample numbers so that random samples may be conducted to continuously check for disease, both by internal personnel and local agricultural authorities.

All Phases should be checking for physical symptoms of disease during biweekly quality checks and feeding. If at anytime a hives looks suspicious it should be quarantined and tested by the QC department. Random samples will be collected and tested regularly in all phases of the bumblebee rearing department.

APPENDIX D: Healthcertificate

JENNIFER M. GRANHOLM
GOVERNOR

STATE OF MICHIGAN
DEPARTMENT OF AGRICULTURE
LANSING

MITCH IRWIN
DIRECTOR

CERTIFICATE OF HEALTH

Date Inspected: February 2, 2006 Number: KB-06-75-AJ-1Qa

To the Animal Health Organization of MÉXICO

This is to certify that insects of the genus and species *Bombus impatiens* described below have been inspected according to appropriate procedures and are considered to be free of Nosema, Varroa Mite, Mellitobia spp., Galleria mellonella and other fungus diseases and insect pests; and that they are considered to conform with the current zoosanitary and ecological requirements of the importing country.

* *

Exporter:	Consignee:
Koppert Biological Systems 28465 Beverly *Romulus, Michigan 48174*	Koppert Mexico S.A. de C.V. Av. Del Marques #38-9 *Parque Industrial Bernardo Quintana* *Municipio del Marques, QUERETARO* *C.P. 76246. MÉXICO*

Number of *Bombus impatiens* queens declared: ____________________

Number and description of packages: ____________________

Place of origin:	*Michigan*
Declared means of conveyance:	*Air Freight*
Point of entry:	*Mexico City*
Zoosanitary permit presented:	113-039-20047, expires February 10, 2006

* *

Date of Issue: February 2, 2006

Name of Authorized Officer (print): Ahmed Jama

Signature of Authorized Officer: ____________________

No financial liability shall attach to the State of Michigan or to any officer or representative of this department with respect to this certificate.

CONSTITUTION HALL • P.O. BOX 30017 • LANSING, MICHIGAN 48909
www.michigan.gov • (517) 373-1104

APPENDIX E: Grower pollination contract

This agreement is made between ,and Koppert Biological Systems Inc.

I. TERM OF AGREEMENT. The term of this agreement shall be for the 2007 growing season.

2. RESPONSIBILITIES OF KOPPERT BIOLOGICAL SYSTEMS:
Koppert shall supply the grower with hives (colonies) of bumble bees to be delivered to the following address:

Introduction week:

b. Koppert provides colonies of the following minimum standards:
- Disease-free colonies evidenced by Michigan Department of Agriculture certification.
- QUAD containing 4 individual hives. Minimum of 1000 bees per QUAD.

Koppert agrees to open and demonstrate the strength of colonies randomly selected by the grower.

3. RESPONSIBILITIES FOR THE GROWER:

1. To obtain a permit from CDFA for the use of *Bombus impatiens* bumble bees.
2. To place each individual QUAD within 3 ft. of a tall structure or tree.
3. To utilize the ‘bee home system’ as explained in the directions for use to collect all bees back into the QUAD after the 5th week of pollination or prior.
4. To dispose of the QUAD and all its content (bees) by drowning, burning or freezing after the 5th week of pollination or prior.
5. To contact Koppert with the dates and quantities of QUAD disposed after use.
6. To exclusively use the QUAD south of the San Fransisco – Reno line (39° latitude).

Koppert can only provide bees between the dates of January 1st and May 31st any QUAD use needs to be planned between those dates.

The grower agrees to pay for _______ QUAD or _______ colonies of bees at the rate of $_______ per QUAD or $_______ per colony.

- A 15% deposit is due upon order confirmation by Koppert.
- Total remaining payment terms are 30 days net; with a credit of 2% on all payments received by close of business on the second day after receipt of invoice.
- Prices ex-warehouse Romulus, MI., shipping cost will be invoiced at cost as a separate line item on the invoices.
- Koppert will be responsible for shipping unless agreed otherwise.
- All complaints or remarks need to be reported to Koppert Biological Systems within 48 hrs. of receipt of the hives.
- All taxes are extra where applicable.

For Koppert Biological Systems, Signed for

Koppert Biological Systems, Inc.
Tel: 1 734 641-3763 or Fax: 1 734 641-3793